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Encapsulation of Eggs by the Rocky Shore Marine Gastropod *Nucella emarginata*: Costs and Benefits of Variation in Capsule Form.

by

Timothy Alexander Clarke Rawlings ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Biological Sciences

Edmonton, Alberta

Fall, 1995

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ABSTRACT

Representatives of many plant and animal taxa enclose their embryos within some form of protective structure. Inter- and intraspecific differences in the morphology of these egg coverings may have profound effects on the development and survival of encapsulated embryos, yet in many taxa little is known about the causes or potential consequences of this variation. Marine prosobranch gastropods are an ideal group to examine the benefits and costs associated with differences in the form of egg coverings. Neogastropod mollusks deposit embryos within a stunning variety of egg capsules, the form of which can vary dramatically within families, among closely-related species, and even among populations of a single species. The adaptive significance of this variation, however, remains largely unknown.

I examined the adaptive significance of intraspecific variation in the morphology of egg capsules of the Northeastern Pacific rocky shore gastropod, *Nucella emarginata*. My objectives were: 1) to document variation in the size, shape, and wall thickness of egg capsules over a wide range of populations, 2) to determine the extent to which these intraspecific differences in capsule form were environmentally or genetically-based, and 3) to examine specific benefits and costs associated with capsules of different form.

Egg capsules of *Nucella emarginata* varied extensively in capsule size, shape, and wall thickness among populations in Barkley Sound. Although capsule form varied among years, the general pattern among populations remained the same. Hence, site-differences were a real and regular phenomenon. Common garden laboratory experiments confirmed that differences in capsule size, shape, and wall thickness among populations were largely environmentally-induced, since capsules produced by laboratory-reared snails tended to converge upon a common phenotype. Because some differences in the size, relative plug length, and wall thickness of capsules remained among populations of laboratory-reared snails, however, variation in capsule form did appear to have some genetic basis.

Variation in some features of *N. emarginata* egg capsules, most notably capsule wall thickness, had a profound effect on the survival of encapsulated embryos. Changes in capsule wall thickness significantly affected the vulnerability of embryos to intertidal predators and ultraviolet radiation. Under certain environmental conditions, thick-walled capsules also had lower rates of water loss compared to thin-walled capsules, although the effect of wall thickness was not large relative to variation in other capsule properties. The benefits of thicker capsule walls, however, also imposed significant costs. Thick-walled capsules were associated with an increased amount of capsular material per unit capsule wet weight, and a reduced density of embryos. Experimental manipulations also revealed that thick walls prolonged the development of encapsulated embryos, possibly due to constraints associated with the diffusion of oxygen or metabolic wastes across the capsule case. Thus, because capsule wall thickness in *N. emarginata:* 1) varied substantially among populations, 2) was at least partly heritable, and 3) was associated with significant benefits and costs, thick-walled capsules appeared likely to represent an adaptive response to specific environmental risks. Given the apparent underlying genetic basis associated with interpopulation variation in capsule body length and relative plug length, variation in these features of *N. emarginata* capsules may also have an adaptive basis. The results of this study thus demonstrate the tremendous effect that subtle differences in capsule form can have on the development and reproductive success of one species of marine gastropod, and provide the rationale for a much broader investigation into the adaptive significance of spawn diversity within the Gastropoda.

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CHAPTER 1

General Introduction

Life-histories of benthic marine invertebrates

Benthic marine invertebrates exhibit a remarkably diverse array of reproductive patterns (e.g., Thorson, 1936; 1950; Mileikovsky, 1971; Chia, 1974; Shuto, 1974; Jablonski and Lutz, 1983). These developmental patterns can generally be categorized as one of four main types (see Thorson, 1950; Mileikovsky, 1971[1]; Vance, 1973): 1) planktotrophic development, involving the production of small pelagic larvae that spend long periods (weeks to months) feeding on suspended particulate material in the water column; 2) pelagic lecithotrophic development, in which pelagic non-feeding larvae are provisioned with their own nutritive reserves and spend only a short duration (hours to days) within the plankton; 3) non-pelagic lecithotrophic development[2], involving the parental brooding of non-feeding larvae, or the enclosure of embryos within egg capsules or masses with associated food reserves; and 4) viviparity, a less common mode of development, in which embryos develop entirely within the body of the adult. Within many phyla, planktotrophic development is believed to be the ancestral mode of development (e.g., Jägersten, 1972; Strathmann, 1978; Strathmann, 1985; but see Haszprunar et al., 1995). Similarities in the form of planktotrophic larvae within oligomerous and spiralian lines, for instance, suggest that within each group planktotrophic larval types have descended from a common ancestor with a feeding, free-swimming larval form (Strathmann, 1978). Likewise, because complex larval feeding structures once lost are rarely regained (Strathmann, 1978), there is strong unidirectional bias in the direction of change from planktotrophic development towards reproductive patterns associated with a reduction or loss of larval feeding structures (e.g., pelagic and non-pelagic lecithotrophy; Strathmann, 1978; 1985).

Why have both pelagic and non-pelagic lecithotrophs evolved independently in several different phylogenetic lines? The evolution of reproductive patterns in benthic marine invertebrates appears to have been influenced profoundly by selective forces favoring the increased survival of developing larvae (e.g., Thorson, 1950; Vance, 1973; Chia, 1974). Theoretical studies examining the fitness-related benefits of one developmental mode versus another, predict that derived developmental modes such as pelagic and non-pelagic lecithotrophy may have evolved in response to adverse environmental conditions within the water column, including low food availability, low water temperatures, and high predation intensity (see Vance, 1973; Christiansen and Fenchel, 1979; Pechenik, 1979; Grant, 1980; Caswell, 1981). Under such conditions, pelagic lecithotrophic development may incur a substantial advantage relative to planktotrophic development, because larvae are independent of the plankton as a source of nutrition, and have considerably shorter planktonic development times. Likewise, non-pelagic lecithotrophic development types, such as brooding or the encapsulation of embryos within benthic egg masses, may also be adaptive under these conditions if the intensity of planktonic predation is greater than within benthic marine habitats (Vance, 1973). These theoretical models, although controversial (see Underwood, 1974; Vance, 1974; Strathmann, 1976), have been helpful a) in providing testable hypotheses concerning the adaptive significance of these reproductive patterns under differing environmental conditions, and b) in interpreting geographic variation in development types associated with gradients in latitude and water depth (e.g., Vance, 1973; Christiansen and Fenchel, 1979; Pechenik, 1979; Grant, 1980; Caswell, 1981; Strathmann, 1985; Clarke, 1992).

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[1] Mileikovsky also recognizes an additional type of larval development termed demersal development

[2] Because of the confusion associated with the term "direct development" (see McEdward and Janies, 1993), I use the term "non-pelagic lecithotrophic" development.

Although these theoretical models have provided the basis for understanding the evolution of different development patterns, we still know very little about the adaptive significance of variation that exists *within* these generalized development types. For instance, intense planktonic predation may have selected for the deposition of eggs within benthic egg coverings, however, this cannot explain the tremendous diversity in the form of encapsulating structures produced by marine invertebrates. Although some of this variation undoubtedly reflects differences in the phylogenetic history of these organisms, substantial differences are apparent even within closely-related groups (e.g., D'Asaro, 1988, 1991, 1993). Differences are evident not only in the morphology of these coverings, but also in their strength (Perron, 1981) and the amount of reproductive energy invested within these structures (Perron, 1981; Perron and Corpuz, 1982). What are the causes of this variation, and what are the consequences of placing embryos within one type of covering versus another? Clearly, answers to such questions are critical to understanding the adaptive significance of variation in the type of developmental patterns exhibited by non-pelagic lecithotrophs.

Egg capsules and masses of marine gastropods

Although the deposition of eggs within benthic egg capsules or gelatinous masses is a common phenomenon among such phyla as the Platyhelminthes, Nemertinea, Annelida, and Mollusca, the diversity of egg coverings produced by marine gastropods remains unrivaled by any other marine organisms. These egg coverings take a variety of forms (see Amio, 1963; Robertson, 1974), such as: 1) the jelly-like masses and ribbons of some trochoidean gastropods (e.g., Hadfield and Strathmann, 1990; Hickman, 1992), 2) the egg capsules and external gelatinous coverings of various caenogastropods (e.g., Houbrick, 1973; Reid, 1990), opisthobranchs (Hurst, 1967; Thompson, 1967) and marine pulmonates (Russell-Hunter et al., 1972; Strathmann, 1987), 3) the spicule-impregnated capsules of neritimorph snails (Andrews, 1935; Houston, 1990), and 4) the tough, leathery capsules of higher caenogastropods (e.g., Thorson, 1935; Bandel, 1973; D'Asaro, 1991, 1993). Since these egg coverings are largely produced by specialized glands in the maternal reproductive tract (see Fretter and Graham, 1962), they are not homologous to envelopes produced by the ovum or developing zygote (Pechenik, 1986). Thus, because extant groups of lower prosobranch gastropods have only simple reproductive systems with no specialized glands for producing capsules or gelatinous secretions, these coverings appear to be a derived condition within the Gastropoda.

The encapsulation of eggs within benthic egg capsules and gelatinous egg capsules may have evolved in response to adverse pelagic conditions, yet these elaborate egg coverings are clearly more than simple bags that confine developing embryos. Gastropod egg capsules and masses can be morphologically very complex, and are often composed of many different laminae secreted from specialized regions of the reproductive tract (e.g., Hurst, 1967; Robertson, 1974; Strathmann, 1987; D'Asaro, 1988; Hickman, 1992). In some species, additional material may also be added to gastropod spawn from glands associated with other parts of the body (e.g., ventral pedal gland; see Bayne 1968a; Sullivan and Maugel, 1984). The form of these egg coverings may thus reflect a new suite of selective pressures acting upon encapsulated embryos (see Pechenik, 1986).

Some anecdotal evidence supports the contention that these structures may be well adapted to the specific environments in which they are deposited. The independent movement of neritimorph, caenogastropod, and pulmonate snails into terrestrial environments, for instance, is associated with the incorporation of calcium into their egg coverings (Tompa, 1976; 1980), a feature extremely uncommon in the egg coverings of exclusively subtidal marine gastropods (but see Ponder, 1970). Likewise, the eggshell structure of some pulmonate gastropods has converged remarkably in form with the eggshells of terrestrial vertebrates, presumably as an adaptive response to a common terrestrial habitat (Tompa, 1984). Although the addition of calcium into gastropod egg coverings undoubtedly reflects its requirement by developing embryos during shell production, calcium may also be important in providing structural rigidity to these protective coverings, as well as protection from desiccation stress in terrestrial habitats

(Bayne, 1968b). The functional significance of many aspects of gastropod egg capsules and egg masses remains unexplored, however.

If gastropod egg coverings are "protective", what are they protecting developing embryos from, and how do differences in the form of these coverings reflect differences in their ability to protect developing embryos? Surprisingly little is known about the precise morphological and physiological consequences of these structures. The few studies that have examined the protective nature of egg coverings have focused on the tough, multi-laminated egg capsules of neogastropods, a derived group of caenogastropods (see Ponder, 1973). Although all neogastropods enclose their embryos within some form of leathery egg capsule (Ponder, 1973), these structures can differ markedly in form, even within families (Thorson, 1935; D'Asaro, 1970; 1988; 1991; 1993; Robertson, 1974; Strathmann, 1987), among closely-related species (Ostergaard, 1950; Perron, 1981; Perron and Corpuz, 1982; Palmer et al., 1990; Collins et al., in review) and among populations of a single species (Rawlings, 1990).

Despite their complex morphology, neogastropod egg capsules are clearly not impervious to many predators (e.g., MacKenzie, 1961; Haydock, 1964; Emlen, 1966; Phillips, 1969; Spight, 1977; Brenchley, 1982; Race, 1982; Martel et al., 1986; Rawlings, 1990), nor are they impermeable to water (Bayne, 1968b; Pechenik, 1978) or solute exchange (Pechenik, 1982, 1983; Hawkins and Hutchinson, 1988; Roller and Stickle, 1989). Nevertheless, capsule walls can reduce the vulnerability of developing embryos to some predators (e.g., Spight, 1977; Brenchley, 1982; Rawlings, 1990), to desiccation (Bayne, 1968b; Pechenik, 1978) and salinity stress (Pechenik, 1982; 1983; Roller and Stickle, 1989), and to attack by bacteria and protists (Pechenik et al., 1984; Lord, 1986). The effect of variation in capsule form on the resistance of developing embryos to specific sources of embryonic mortality, however, has rarely been examined (but see Bayne, 1968b; Perron, 1981).

Although differences in the morphology of gastropod egg capsules are most apparent among species of neogastropods, intraspecific comparisons provide a valuable tool for teasing apart the causes and potential consequences of variation in capsule form. Unlike interspecific comparisons, which may potentially be subject to confounding phylogenetic effects, interpopulation variation in capsule form may reflect local adaptation to specific environmental conditions, particularly in those species with no pelagic larval stage, and hence low gene flow among populations. If such variation has a heritable basis, and differences in capsule form affect the survival of embryos, then those selective agents that have influenced the evolution of capsule form within marine gastropods may be identified.

Natural history of *Nucella emarginata*

In this thesis, I examine the adaptive significance of variation in capsule form among populations of the marine intertidal gastropod, *Nucella emarginata* (Deshayes, 1839; northern species[3]; Palmer et al., 1990; Prosobranchia: Muricidae). These neogastropods are common inhabitants of rocky intertidal shores from California to Alaska and range across wide extremes in wave exposure. Snails spawn erect, vase-shaped egg capsules year-round within the mid-high region of the intertidal zone (Strathmann, 1987; Fig. 1-1). Within these capsules, embryos feed on nutritive nurse eggs and possibly albumen (Rawlings, pers. obs.) during a developmental period lasting up to 140 days (Palmer, 1994), before emerging from the capsule chamber as juvenile snails. Because these larvae lack a pelagic stage, gene flow among geographically-separated populations may be low (e.g., Grant and Utter, 1984; Palmer, 1984; Day, 1990). As a consequence, populations may have become adapted to local environmental conditions (see Yamada, 1989). Thus, given that the morphology of *N. emarginata* capsules varies extensively among three populations examined in Barkley Sound, British Columbia (Rawlings, 1990), this variation may reflect adaptive responses to local differences in the intensity of environmental stresses acting upon developing embryos.

Egg capsules of *Nucella emarginata*

The production of *Nucella* egg capsules, like capsules of most other neogastropods, is a complex process (Fretter and Graham, 1962). Capsular material is secreted within the lumen of a bilobed capsule gland in the pallial region of the female's oviduct. Following the deposition of eggs and albumen within this secretion, the capsule leaves the pallial oviduct in the form of a soft pliable bag filled with developing embryos. This soft capsule is then transferred via a temporary groove in the propodium to the ventral pedal gland in the base of the foot, where it is molded into its final form and hardened, possibly through a process of sclerotization (see Price and Hunt, 1973; 1974; 1976). The final sculptured capsule consists of three main regions: a central chamber housing developing embryos, an apical mucoid plug that seals the chamber from the external environment and acts as an escape hatch for juvenile snails, and a short stalk that attaches the chamber to the substratum (Fig. 1-1). Two thickened seams are also present on either side of the capsule chamber. These reflect the junction points of the two lobes of the capsule gland (see Fretter and Graham, 1962).

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[3] Because of the recent discovery of two morphologically very similar species within the current taxon *Nucella emarginata* (Palmer et al., 1990), there is some question as to which of these species will retain the name *emarginata*. This complication has arisen because of confusion regarding Deshayes' original description of the type locality and type specimen of *Nucella emarginata*. Until this matter is resolved, Palmer et al. (1990) have advocated that these two species be referred to as *N. emarginata* (northern) and *N. emarginata* (southern). Hence, because this study was conducted within the range of the putative "northern species", this species will be referred to as *Nucella emarginata* (northern species).

The walls of *N. emarginata* capsules consist of three laminae resolvable using light microscopy (Rawlings, 1990): 1) an outer lamina (< 5 µm thick) that seals the capsule wall from the external surroundings (see D'Asaro, 1988), 2) a thick middle lamina, variable in thickness, consisting of three separate layers, and 3) a thin transparent inner lamina[4] (< 6 µm thick), that surrounds developing embryos and intracapsular fluid. This innermost lamina appears to be connected to and composed of the same material as the mucoid plug. Although the inner two laminae are secreted by the capsule gland, the outermost lamina may be a secretion of the ventral pedal gland in this genus (see Bayne, 1968a). This arrangement of capsule laminae appears to be quite typical of muricid gastropods (D'Asaro, 1988), although the actual appearance and composition of each lamina can differ considerably among species within this genus (Fretter and Graham, 1962; Bayne, 1968a; Rawlings, pers. obs.).

Nucella egg capsules, like most neogastropod egg capsules examined so far, are composed mainly of protein and carbohydrate (Bayne, 1968a; Flower et al., 1969; Hunt, 1966; 1971; Flower, 1973; Price and Hunt, 1973; Sullivan and Bonar, 1984; Colman and Tyler, 1988; Hawkins and Hutchinson, 1988), with some evidence for the presence of bound lipids within the capsule wall (Price and Hunt, 1974; Colman and Tyler, 1988). Extensive studies of capsules of the neogastropod whelk, *Buccinum undatum*, for instance, indicate that amino acids account for 78% of the total weight of the capsular material. The capsule proteins of this species and the muricid snail, *Urosalpinx cinerea*, both have fibers with well-defined α - helical conformations that are packed into ribbon-like units (Tamarin and Carriker, 1967; Flower et al., 1969). The chemical composition of these proteins suggests a similarity between capsule walls and certain keratin structures (Price and Hunt, 1973). The inert nature of the capsule walls and their resistance to chemical treatment also indicate that these proteins may be stabilized by extensive cross-linking, perhaps as the result of tanning (Hunt, 1971; Price and Hunt, 1973; 1974; 1976). Detailed histochemical analyses of the egg capsules of *N. lapillus* and other neogastropod capsules (e.g., *Nucella lapillus*, Bayne, 1968a; *Ilyanassa obsoleta*, Sullivan and Maugel, 1984; *Colus jeffreyianus*, Colman and Tyler, 1988; *Ocenebra erinacea*, Hawkins and Hutchinson, 1988) have shown, however, that the chemical composition of the capsule wall is not homogeneous throughout. Different wall laminae have different chemical components, and thus, may have different functions.

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[4] Using transmission electron microscopy, D'Asaro, 1988 has shown that this lamina is actually composed of two distinct laminae in most muricid capsules.

Objectives

Although a considerable amount is known about the general ecology and reproductive biology of *Nucella emarginata* (e.g., Emlen, 1966; Spight and Emlen, 1976; Spight, 1977; Palmer, 1980; Gosselin, 1994), little is known about the functional properties of their egg capsules. The specific objectives of this thesis therefore were three-fold: 1) to document variation in the size, shape, and wall thickness of *N. emarginata* capsules over a wide range of populations, 2) to determine the extent to which these intraspecific differences in capsule form were environmentally or genetically-based, and 3) to examine some specific benefits and costs associated with capsules of different form.

This research was conducted at the Bamfield Marine Station and in the neighboring Deer Group Islands. Intraspecific comparisons of capsule form were made among ten different populations of *N. emarginata* separated along a gradient in wave-exposure in Barkley Sound, British Columbia (Fig. 1-2).

Organization of this thesis

This thesis is divided into eight chapters, including this introductory chapter (Chapter 1), and a general summary chapter (Chapter 8). Because chapters 2 through 7 are written as separate, publishable units, some repetition of text and data has been unavoidable. Summaries of each chapter are as follows:

Chapter 2. In this chapter, I increase the scope of a preliminary study of intraspecific variation in the form of *N. emarginata* capsules (Rawlings, 1990) by 1) documenting variation in capsule size, shape, and wall thickness among ten populations of *N. emarginata* separated along a gradient of wave-exposure and 2) examining the stability of these patterns of intraspecific variation over time. Also, to understand the adaptive significance of interpopulation differences in capsule form, I determine the extent to which these site-differences are environmentally or genetically-based. The variation in capsule morphology recorded in this chapter provides the basis for comparisons of the benefits and costs of differences in capsule form examined in later chapters (Ch. 3 to Ch. 7)

Chapter 3. Because the capsule wall of *N. emarginata* capsules represents the only physical barrier separating developing embryos from their external environment, and these snails exhibit no form of parental defense, the thickness of the capsule wall may reduce the vulnerability of embryos to specific environmental risks. To assess the significance of the extensive variation in capsule wall thickness observed among populations of *Nucella* *emarginata* (Rawlings, 1990), I examine the resistance of thick- and thin-walled capsules to predation by the isopod *Gnorimosphaeroma oregonense* (Dana, 1852). These isopods are abundant intertidal organisms, are known to feed on *N. emarginata* capsules, and thus, may have a profound influence on the survival of encapsulated embryos.

Chapter 4. Tough, multilaminated capsules may be one means of protecting embryos from physical stresses associated with a benthic intertidal habitat. Since many intertidal organisms exhibit adaptive structural, behavioral, and physiological modifications in response to desiccation stress, I investigate the ability of capsule walls to resist water loss. I specifically examine 1) the resistance of *N. emarginata* egg capsules to desiccation under both laboratory and field conditions, and 2) the ability of encapsulated embryos of this species to tolerate extensive water loss from the capsule chamber. In addition, to investigate the relative importance of intraspecific differences in capsule size, shape and wall thickness on desiccation rates, I use a simple desiccation model to simulate the impact of this variation in capsule form on rates of water loss, and the survival of developing embryos.

Chapter 5. Ultraviolet radiation (UV) has rarely been considered a potential source of mortality for encapsulated gastropod embryos, even though natural levels of UV may have profound effects on other marine organisms. In this chapter, I examine the exposure of benthic egg capsules of *Nucella emarginata* to direct solar radiation under natural field conditions, and assess the degree to which capsule walls may protect developing embryos from UV. I also compare the spectral properties of capsules collected from different populations of *N. emarginata*, and from two other local species of *Nucella*.

Chapter 6. Here, I describe a technique that allows developmental changes in the behavior and morphology of muricid embryos to be observed through the capsule wall, and that can also be used to assess both benefits and costs associated with enclosure of embryos within benthic capsules. This technique involves the selective removal of the thick, and often opaque, outer wall laminae of muricid egg capsules, leaving embryos enclosed within a thin-walled transparent barrier. Because stripped capsules lack the thick outer capsule wall, embryonic development within stripped and intact capsules can be compared to assess 1) the benefits of the outer capsule wall in protecting embryos from specific sources of mortality, and 2) their costs in terms of limiting the rate of diffusion of oxygen into and metabolic wastes out of the capsule chamber. I use this technique in Chapter 7.

Chapter 7. Although the size, shape, and thickness of gelatinous egg masses can have substantial effects on the development of embryos embedded within, little is known about diffusive constraints associated with the encapsulation of eggs within the tough, fluid-filled capsules of higher prosobranch gastropods. An increase in the wall thickness of these capsules may provide developing embryos with better protection from potentially lethal environmental stresses, but may also limit the exchange of oxygen, nutrients, and metabolic wastes between embryos and their external environment. In this chapter, I examine diffusive constraints associated with the deposition of eggs within egg capsules of *N. emarginata*. Because the thickness of capsule walls varies significantly among populations of this species, I compare the allocation of embryos within capsules of differing wall thickness. I also examine the development time of embryos within whole and artificially stripped egg capsules, and the influence of environmental conditions, including water motion and aerial exposure, on the rate of embryonic development.

Chapter 8. In this concluding chapter, I briefly discuss the evolution of egg coverings within the Gastropoda, comment upon what is known about the diversity of spawn types that have evolved within this group, and illustrate how direct tests of the adaptive value of specific capsule traits are critical to understanding the evolutionary significance of this variation in form.

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Figure 1-1. a) Seam and side views of a *Nucella emarginata* egg capsule illustrating the three main regions: a central chamber housing developing embryos, an apical mucoid plug that seals the chamber from the external environment and acts as an escape hatch for juvenile snails, and a short stalk that attaches the chamber to the substratum. The capsule body refers to the length of the capsule chamber and plug (drawings modified from D'Asaro, 1991). b) Side view of a freshly-deposited *N.emarginata* egg capsule with part of the outer wall removed to illustrate the large number of eggs (mostly nutritive nurse eggs) packaged within the capsule chamber. c) Side view of a capsule with part of the outer wall removed illustrating the packaging of early 4th-stage veligers within the chamber; all nurse eggs have been consumed. Scale bars = 1 mm.

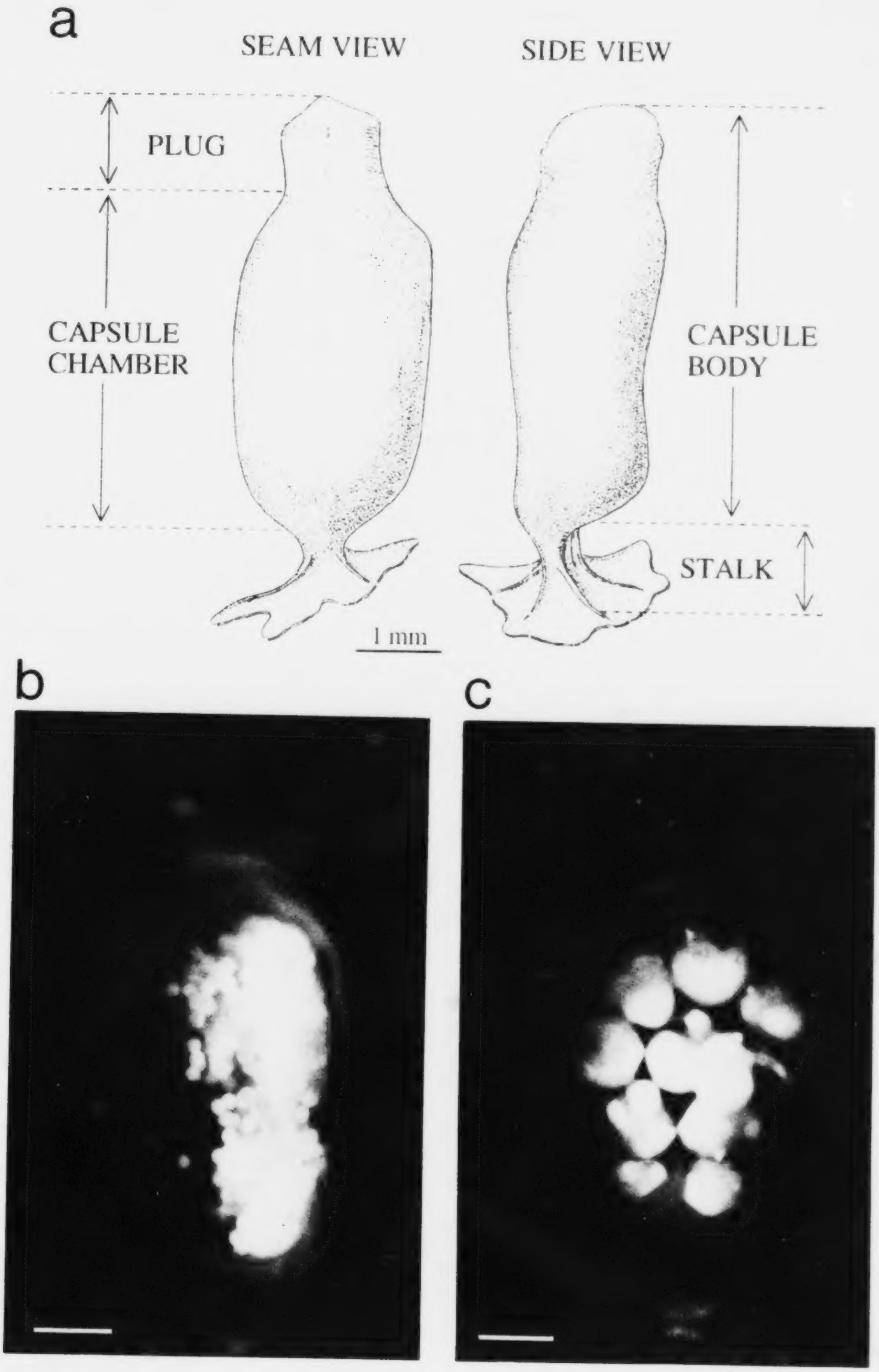
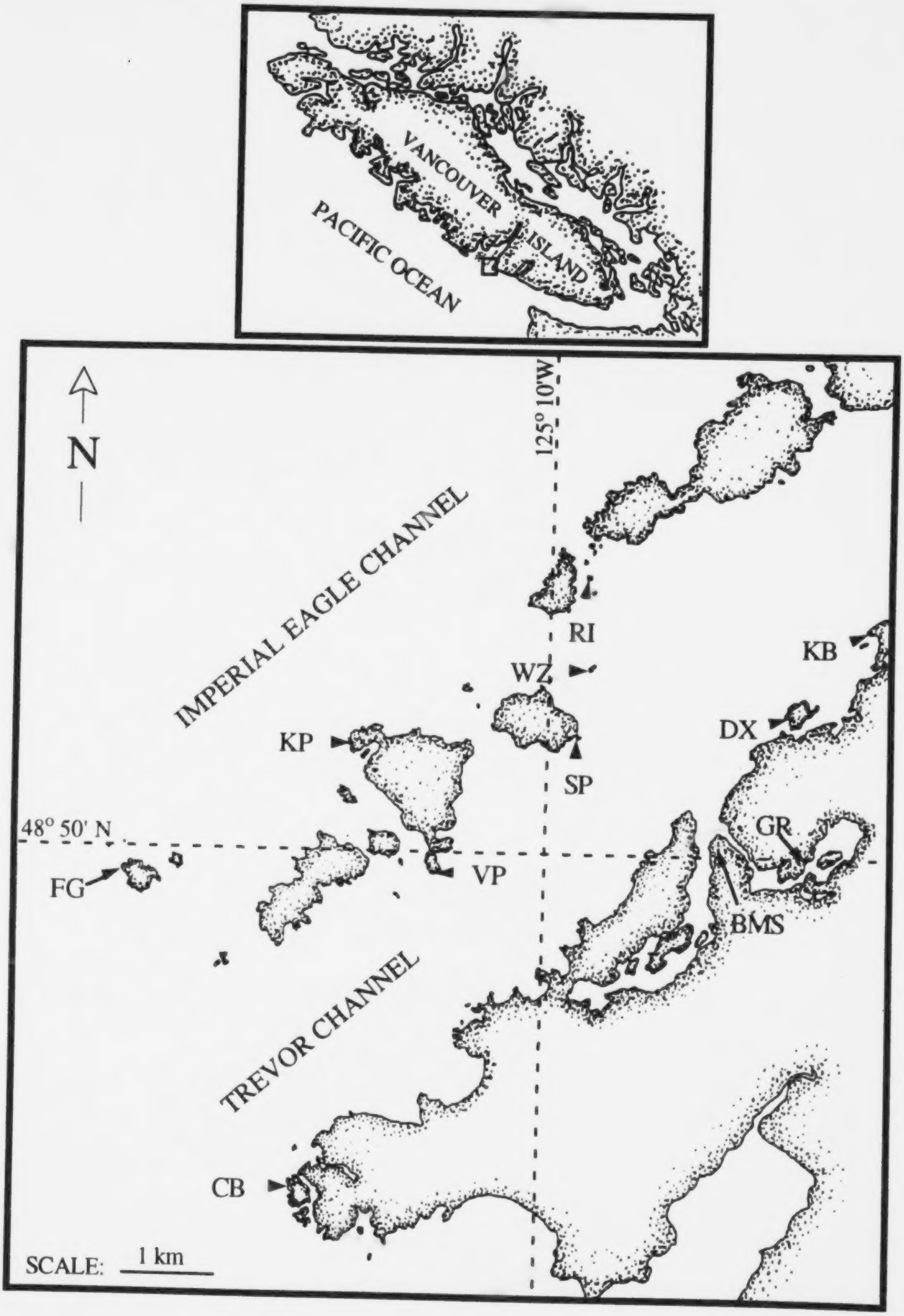

a
SEAM VIEW
SIDE VIEW
PLUG
CAPSULE CHAMBER
CAPSULE BODY
STALK
1 mm
b
c

Figure 1-2. Map of the Deer Group Islands in Barkley Sound on the west coast of Vancouver Island, British Columbia. The locations of the ten populations of *Nucella emarginata* examined in this thesis are marked by arrows. Abbreviations refer to: GR = Grappler Inlet, RI = Ross Islet, KB = Kelp Bay, DX = Dixon Island, SP = Self Point, WZ = Wizard Rock, VP = Voss Point, KP = Kirby Point, CB = Cape Beale, and FG = Folger Island. BMS indicates the location of the Bamfield Marine Station.


VANCOUVER
ISLAND
PACIFIC OCEAN
N
125° 10'W
IMPERIAL EAGLE CHANNEL
RI
KB
WZ
DX
KP
SP
48° 50' N
GR
FG
VP
BMS
TREVOR CHANNEL
CB
SCALE: 1 km

CHAPTER 2

Genetic and environmental components of variation in reproductive characteristics among populations of the rocky shore gastropod *Nucella emarginata*

Abstract

Although marine gastropods produce a stunning variety of egg capsules and gelatinous egg masses, the adaptive significance of these varied structures remains elusive. Species exhibiting intraspecific variation in the morphology of their egg coverings, however, provide an ideal opportunity to examine the causes and potential consequences of enclosing embryos within capsules or masses of different form, since direct comparisons of spawn types can be made without the concern of confounding phylogenetic effects. Despite this, few studies have examined variation in capsule form among populations exposed to differing environmental regimes. In the present study, I documented variation in egg capsule morphology among populations of the rocky shore gastropod *Nucella emarginata*. My objectives were three-fold: 1) to compare the size, shape, and wall thickness of capsules among ten populations of *N. emarginata* separated along a gradient of wave-exposure, 2) to examine the stability of these patterns of intraspecific variation over time, and 3) to determine the extent to which these site-differences in capsule form were environmentally or genetically based.

Comparisons of capsule morphology among populations revealed substantial differences in capsule size, shape, and wall thickness. Capsules from wave-sheltered shores had large, narrow chambers, with relatively small plugs per unit length of the capsule chamber. In contrast, capsules spawned at wave-exposed sites were significantly smaller in chamber length, and had wider chambers, and proportionately larger plugs, than those from more wave-sheltered shores. Capsule wall thickness also varied significantly among sites; relatively thick-walled capsules were produced at both wave-sheltered and wave-exposed extremes, while substantially thinner-walled capsules were deposited at sites more intermediate in wave-exposure. Although there was some variation in capsule form among years, the general pattern among populations remained the same. Hence, site-differences were a real and regular phenomenon.

Common garden laboratory experiments revealed that site-differences in capsule size, shape and wall thickness were largely environmentally-induced, since capsules produced by laboratory-reared snails tended to converge upon a common phenotype. Because some differences in capsule size, relative plug length, and wall thickness remained among

populations of laboratory-reared snails, however, some components of variation in capsule form did have a genetic basis. Experimental manipulation of environmental conditions, including food ration and predation risk, had no significant effect on the wall thickness of capsules produced, although food ration did have a significant effect on capsule size, the packaging of embryos per capsule, and the number of capsules produced by spawning females.

Although several aspects of capsule form were phenotypically plastic, adaptive explanations for among-population differences are still possible, since the plasticity itself may have a genetic basis. When coupled with the significant benefits and costs associated with the enclosure of embryos with specific morphs of *N. emarginata* capsules, therefore, differences in capsule form observed among populations in this study may represent adaptive, induced responses to specific environmental risks.

Introduction

The deposition of eggs within protective egg coverings is a feature common to many lineages of marine gastropods. These egg coverings can take a variety of forms, such as: 1) the jelly-like masses and ribbons of some trochoidean gastropods (e.g., Hadfield and Strathmann, 1990; Hickman, 1992), 2) the egg capsules and external gelatinous coverings of various caenogastropods (e.g. Houbrick, 1973; Reid, 1990), opisthobranchs (Hurst, 1967; Thompson, 1967) and marine pulmonates (Russell-Hunter et al., 1972; Strathmann, 1987), 3) the spicule-impregnated capsules of neritid snails (Andrews, 1935; Houston, 1990), and 4) the tough, leathery capsules of neogastropods (e.g., Thorson, 1935; Bandel, 1973; D'Asaro, 1991, 1993). Despite the tremendous diversity of egg coverings that have evolved within the Gastropoda, however, little is known of the consequences of enclosing embryos within these varied structures. Although the robust egg capsules of neogastropods and neritid snails undoubtedly provide protection, the effectiveness of these structures in defending embryos from specific environmental risks has been examined for relatively few species (see review in Pechenik, 1986). Likewise, few studies have investigated the role of the egg mass jelly of various caenogastropods, opisthobranchs, and marine pulmonates. Gelatinous egg masses may protect developing embryos from predation via chemical defensives in the egg mass jelly (Pawlik et al., 1988), and possibly from desiccation (Fretter and Graham, 1962; Reid, 1990). However, these egg masses may also serve to provide embryos with a source of nutrition during development (Clark and Goetzfried, 1978) and inducers for metamorphosis (Gibson and Chia, 1994). Clearly, therefore, we still have a very superficial understanding of the role of encapsulating structures in the life-histories of benthic marine gastropods.

The form of egg coverings can vary markedly even within specific clades of marine gastropods (e.g., Bandel, 1973; D'Asaro, 1991, 1993; Reid, 1990, 1991; Collins et al., submitted). Snails within mesogastropod families such as the Littorinidae, for instance, produce a diversity of spawn types unrivaled within the Gastropoda. Within the genus *Littorina* alone, snails can spawn entirely pelagic egg capsules, capsules deposited within benthic jelly masses, and unencapsulated eggs embedded in a jelly matrix. Others have foregone the production of extraembryonic materials entirely by retaining embryos within a brooding chamber in the oviduct (Reid, 1990). Neogastropod molluscs also produce a startling array of capsule forms; capsule size, shape, surface sculpture, and wall thickness can vary within families of this group (D'Asaro, 1988, 1991, 1993), among closely-related species (Ostergaard, 1950; Perron, 1981; Collins et al., in review), and even among populations of the same species (Rawlings, 1990). Recently, adaptive explanations have been proposed for apomorphic spawn types within prosobranch gastropods by mapping spawn characters onto phylogenies of these groups (e.g., *Littorina*, Reid, 1990, 1991; *Nucella*, Collins et al., submitted). Using this approach, Reid (1990) has suggested that geographic trends associated with capsule size and the packaging of eggs within pelagic egg capsules of *Littorina* spp. may have resulted from the greater severity of predation by planktonic organisms in higher latitudes. As yet, this hypothesis has not been tested. Nevertheless, comparative techniques (sensu Harvey and Pagel, 1991), coupled with experimental tests of the functional significance of these spawn types, are critical to understanding the diversity of egg coverings that exists within the Gastropoda.

Given that even closely-related species of marine gastropods can produce very different spawn types, how functionally significant is this variation? First, the production of extraembryonic products, such as capsular cases and gelatinous egg masses, can represent an extreme form of energetic investment relative to free spawning gastropods with no form of parental care. This expenditure of energy may not be trivial. Capsular cases of *Conus pennaceus*, for instance, account for 37% of the energy allocated to whole capsules (i.e., capsular cases plus intracapsular fluid and eggs; Perron, 1981; Perron and Corpuz, 1982). Hence, the production of capsules walls may divert a substantial amount of energy away from the production of eggs. Second, small changes in the size, shape, and thickness of egg capsules and masses can affect the rate of diffusion of oxygen and metabolic wastes into and out of the capsule chamber (Chaffe and Strathmann, 1984; Strathmann and Chaffee, 1984; Strathmann and Strathmann, 1995; Cohen and Strathmann, submitted; Chapter 7). Consequently, changes in morphology may place limits on the number of embryos that can develop successfully within an egg mass or the developmental rate of those embryos. Finally, two recent studies have demonstrated that subtle differences in capsule morphology can have a significant impact on the development and survival of encapsulated embryos. Perron (1981) found that capsule wall strength and the proportion of energy invested in capsular cases among closely-related species within the genus *Conus* were directly related to the encapsulated development times of embryos, such that embryos with protracted development were enclosed within stronger, thicker-walled and energetically more expensive capsules. Thus, in species with long-term embryonic development, the costs of increased energy expenditure may be offset by the benefits of the increased protective quality of tough, thicker walled capsules (Perron, 1981; Perron and Corpuz, 1982). Likewise, thicker walled capsular cases of *Nucella emarginata* significantly reduced the vulnerability of embryos to specific intertidal predators in laboratory experiments (Rawlings, 1990; Chapter 3). Hence, intra- and interspecific differences in the morphology of egg coverings may have profound consequences for developing embryos.

Although differences in the morphology of gastropod egg capsules are most apparent among species, intraspecific comparisons can be a valuable tool for teasing apart the causes and potential consequences of variation in capsule form. In species with no pelagic larval stage, and low gene flow between populations, variation in capsule form among populations may reflect local adaptation to specific environmental conditions. To demonstrate the adaptive significance of differences in capsule form, therefore, one must 1) examine the genetic basis of interpopulation differences in capsule morphology, since some portion of this variation must be heritable if the capsule form is to be subject to selection, and 2) demonstrate the fitness-related effects associated with differences in capsule morphology.

In the present study I examined the genetic and environmental components of variation in the morphology of egg capsules among populations of the rocky shore marine gastropod, *Nucella emarginata* (Deshayes, 1839)("northern species"; Palmer et al., 1990). This species is an ideal candidate for studies of intraspecific variation in capsule form. First, it is a common inhabitant of intertidal zones ranging from Alaska to California and can be found spanning both wave-sheltered and wave-exposed habitats. Hence, developing embryos may be exposed to a wide variety of environmental risks. Second, egg capsules are deposited year-round within 6-10 mm-long vase-shaped egg capsules and attached to firm substrata in the intertidal zone. Thus, capsules are relatively large, easy to locate, and usually available throughout the year (Strathmann, 1987). Third, like all seven species of northern hemisphere *Nucella*, embryos emerge from their capsules as juvenile snails, and therefore, lack a planktonic larval stage (Collins et al., submitted). As a result, populations tend to be genetically fragmented (see Grant and Utter, 1988; Day, 1990) and thus may be locally adapted to specific environmental conditions (Palmer, 1984; Yamada, 1989). Finally, preliminary comparisons of capsule form among three populations of *N. emarginata* have revealed significant differences in capsule form (Rawlings, 1990), thus providing the groundwork for the present study.

The objectives of this study were threefold: 1) to increase the scope of a preliminary study (Rawlings, 1990) by examining variation in the size, shape, and wall thickness of *N. emarginata* capsules over a much wider range of habitats, 2) to determine the stability of patterns of intraspecific variation over time, and 3) to examine the extent to which these intraspecific differences in capsule form were environmentally or genetically-based. To compare capsule morphology among populations, I examined attributes of *N. emarginata* egg capsules that I felt would be good descriptors of the size and shape of the capsule chamber housing developing embryos, as well as traits that might reflect the protective quality of the egg capsule. Because my initial results indicated that many of these traits were phenotypically plastic, I also attempted to induce changes in the protective quality of capsules by modifying environmental conditions in a common laboratory environment. As previous research has suggested that food availability (McKillup and Butler, 1979) and the presence of capsule-eating predators (Rawlings, 1990) may affect the morphology of capsules produced by different populations of marine gastropods, I examined the potential for these two factors to induce changes in *Nucella emarginata* egg capsules.

Materials and Methods

Field Collection of *N. emarginata* and their Egg Capsules

To examine the extent of variation in the morphology of egg capsules among local populations of the marine intertidal snail, *Nucella emarginata*, I selected ten populations in the vicinity of the Bamfield Marine Station in Barkley Sound, British Columbia (48°50'N; 125°08'W). Collection sites were separated geographically along a gradient of wave-exposure (see Fig. 1-2 in Chapter 1); these locations have been ranked tentatively in wave exposure based on 1) the maximum height of the *Balanus glandula* zone (Darwin, 1854), 2) the lowest height of vascular plants, and 3) the height of approaching swells (Palmer et al., unpub. data). Increased wave exposure is generally correlated with larger swells, an enhanced upward extension of the *B. glandula* zone on the shore (Kitching, 1976; Carefoot, 1977) and a reduced downward extension of the lowest vascular plants (Palmer et al., unpub. data).

Egg capsules were collected chiefly during the summer months, when capsules were present at all populations and access to exposed sites was less restricted by inclement weather. To compare capsule size, shape, wall thickness, and dry weight among populations, I collected ten clutches of freshly-laid capsules from all ten sites in May 1990 and June 1991. Fresh capsules were rarely fouled by encrusting organisms or nibbled by crustacean predators, and could be identified by the appearance of large numbers of nurse eggs through the capsule wall, and the lack of obvious well-developed shelled embryos. Capsules were also collected over the period from April - July 1992 at Grappler Inlet, Ross Islet, Kirby Point and Cape Beale sites. Capsules collected during other times of the year were included in these among-population comparisons where possible.

Capsule size and shape. The size and shape of capsules was compared among populations by selecting three capsules from each of ten clutches that were collected at each site in June 1991. Capsules were measured for body length (not including the stalk), plug length, chamber length, and chamber width (as defined in Chapter 1). Chamber volume was also estimated using the formula for a prolate ellipsoid: $V = (4/3)\pi (a/2)(b/2)^2$, where a and b are measures of the average chamber length and average chamber width, respectively. To determine if these site-differences persisted from year to year, I also compared capsules collected from four of these sites in the summer of 1992 (n = 1 capsule/clutch x 50 clutches).

Capsule wall thickness. The thickness of capsule walls was compared among sites using ten clutches of capsules collected from all sites in 1990 (n = 1 capsule/clutch), and 1991 (n = 3 capsules/clutch). All capsules were marked at a point 70% along the length of the chamber housing developing embryos, and then sectioned at the marked point using a freeze microtome. Capsule walls are thinnest and least variable in this region (see Rawlings, 1990). Capsule sections were then mounted under a compound microscope equipped with an ocular micrometer. Wall thickness was computed as the average of eight measurements recorded around the circumference of the mounted section, using the two seams of the capsule wall as reference points.

To ensure that capsules spawned at different heights in the intertidal zone did not differ in wall thickness, in June 1991 I also collected nine to ten clutches of capsules from the highest and lowest transects along which capsules could be found at Ross Islet and Kirby Point. One capsule from each clutch was collected and sectioned as described above.

Capsule dry weight. The amount of material allocated to capsular cases and to whole capsules was compared among all sites in 1991, and among four sites in 1992. Two capsules were selected from each clutch and were scrubbed lightly to remove any surface debris. Capsules were then measured under a dissecting microscope at 6 - 12 x magnification, and rehydrated in seawater to restore any water lost through desiccation while measuring. Both capsules were then briefly rinsed in distilled water to remove any surface salts, damp dried by wiping them with an absorbent tissue, and weighed to the nearest 0.01 mg using a Mettler digital balance. One capsule of the pair was then placed into an aluminum weighing dish and dried in an oven to constant weight at 70°C (dried whole capsule). The remaining capsule was then completely emptied of its contents by bisecting the capsule with a razor blade, rinsed in distilled water and dried at 70°C (dried capsular case). To correct for the effect of salts within the capsule chamber on capsule wet weight, I subtracted the weight of salts within an equivalent volume of 30‰ seawater for each capsule. This amount was also subtracted from the weight of all dried whole capsules, since the initial wet weight (and hence amount of salts) was known for each capsule.

Laboratory populations of *Nucella emarginata*

In May 1990, I collected 30 adult *N. emarginata* from Grappler Inlet (48°49'54" N, 125°06'54" W), Ross Islet (48°52'12" N, 125°06'54" W), and Folger Island (48°49'48" N, 125°15'00" W) to establish three laboratory populations of snails. These laboratory populations were used to determine if site-differences in capsule morphology persisted once field-collected snails had been brought into a "common" laboratory environment, and also to compare the morphology of capsules produced by F_1 progeny of these snails (i.e., snails that had been born and raised entirely within the laboratory). To establish these breeding groups, snails were measured for shell length and then sorted according to sex. For each population, one reproductively mature male and female *N. emarginata* were allocated to each of 10 mesh-paneled containers. Snails were provided with an excess supply of barnacle-covered rocks (*Balanus glandula*, Darwin, 1854) for food, and immersed in a constant flow of running seawater. Each cage was initially checked for newly-laid egg capsules at two-week intervals. Because the frequency of spawning declined in the fall and winter months, spawning checks were reduced to three-week intervals after August 1990. When capsules were present, five capsules were collected from each female snail and preserved in 5% formalin (in seawater) for later measurement. Preservation and long-term storage of capsules in 5% formalin does not have any significant effect on the thickness of the capsule wall (Rawlings, unpub. data). Every six-weeks, cages were cleaned and barnacle rocks replaced with freshly collected rocks from the field.

F_1 Generation

Excess capsules laid by laboratory breeding groups of *Nucella emarginata* during June - August 1990 were used to raise an F_1 generation of laboratory snails. I collected capsules at regular spawning checks from three parental crosses within each laboratory group. Each clutch of capsules was then placed in a mesh-paneled bag (see Palmer, 1984) and suspended in a shallow seawater table. Once embryos neared completion of their development, capsules were transferred to large cages with 500 μm Nitex panels. Hatchling snails which emerged from these capsules were raised following the procedure described by Palmer (1984). In March 1991, at a shell length of <15 mm, I sexed all snails and placed them in sex- and family-specific holding cages (15 male or female snails per cage). Cages were cleaned and food replaced every four - six weeks. These snails were used in the following experiments to assess the relative importance of a) genetic versus environmental effects, b) food ration, and c) predator-stimulus, on intraspecific variation in capsule morphology.

a) Genetic versus environmental effects on capsule form

To assess the extent of genetic variation in capsule form, the size, shape, and wall thickness of capsules produced by F_1 snails were compared among laboratory populations. In April 1991, five F_1 female snails from each of two crosses within Grappler, Ross and Folger lines were placed in their own mesh-paneled containers and paired with male F_1 offspring. Snails were kept under the same laboratory conditions as those described previously, and were provided with an excess of barnacle-covered rocks for food. At regular intervals, each cage was checked for capsules. Once snails had started to spawn (Sept/Oct, 1991), three - five capsules were collected from each female snail and preserved in 5% formalin for later measurement.

b) Food Ration

Pretreatment conditions: To examine the effect of food ration on capsule morphology, I collected 27 reproductively mature male and female F_1 snails (nine female snails from each of three different parental crosses) from Grappler Inlet, Ross Islet, and Folger Island lines in November 1991. Each pair of male and female snails was placed in its own mesh-paneled cage, and provided with an *ad libitum* supply of barnacle-covered rocks for food. Cages were monitored for capsules at regular intervals until all females had produced at least one clutch (March 1992). The first five capsules collected during this time interval were preserved in 5% formalin to determine the wall thickness produced prior to the exposure of snails to treatment conditions.

Treatment conditions: Once all snails had produced at least one clutch of five capsules, I assigned sibling female *N. emarginata* in equal numbers to one of three ration treatment conditions: 33% ration, 66% ration, and 100% ration. Ration was manipulated by changing the availability of food at 10 d intervals:

	DIET RATION		
DAYS	33%	66%	100%
0-10	FOOD	FOOD	FOOD
10-20	NO FOOD	FOOD	FOOD
20-30	NO FOOD	NO FOOD	FOOD

At the end of a 30 d period, the cycle was repeated and freshly-collected barnacle rocks were placed in each cage. To ensure that this design actually altered the amount of food consumed, I determined the amount of food eaten per cage in the 33% and 100% ration treatments over 10 d in October 1992. To do this, I removed all barnacle rocks after 10 d exposure to snails within the 33% and 100% ration treatment. The amount of food consumed was determined by measuring the opercular diameter of each drilled *Balanus glandula* under a dissecting microscope at 6 x magnification. Once each barnacle had been measured, it was marked to avoid repeat measuring. The number of barnacles eaten was then converted into ash free dry mass of barnacle flesh consumed using an equation derived by Palmer (1990a): log (ash-free dry mass of barnacle) = 3.285 log (opercular diameter) - 1.439. The amount of food consumed per 10 d period in the 100% ration treatment was multiplied by three to determine the amount consumed over the full 30 d cycle.

At the end of six months exposure to treatment conditions, I began to collect capsules from each female snail to determine the effect of food ration on: a) capsule size and the packaging of embryos within each capsule, b) capsule wall thickness, and c) the dry weight of capsular cases. Because spawning frequency was low at this time of year, especially for snails in the low food ration treatment, capsules were collected over three months. I continued to monitor the capsule production of female snails exposed to different treatment conditions for one full year, after which the experiment was terminated.

Determination of capsule size and the packaging of embryos per capsule: Six capsules were collected from each female to compare the capsule size and the packaging of embryos within capsules from different ration treatments. Because some snails did not spawn over the specified time interval, capsules were only collected from a total of 70 females. Once collected, capsules were placed in mesh bags and suspended in flowing seawater. As soon as developing embryos could be discerned through the capsule wall, and the majority of the nurse eggs had been consumed (4 - 6 weeks), capsules were removed from their mesh bags and measured under a dissecting microscope at 6 - 12 x magnification. Capsules were then opened, by cutting off the capsular plug with a pair of fine dissecting scissors, and the number of embryos within each capsule counted.

Capsule wall thickness: I also selected three capsules from each of five females in the 33% and 100% ration treatments to section for capsule wall thickness (n = 5 females per treatment x 2 treatments per population x 3 populations). Since capsules were collected and sectioned from the same snails under pretreatment conditions, I was able to determine the effect of treatment conditions and adult age on the thickness of capsule walls produced in the laboratory.

Dry weight analysis: Four capsules were collected from each female to measure the dry weight of the capsular case (n = 3 capsules/female) and whole capsule (n = 1 capsule/female) produced in the 33% and 100% food treatments. Because of sporadic spawning by *N. emarginata*, capsules were preserved in 5% formalin until four or more capsules had been collected from all females. This ensured that dry weight measurements were made under identical conditions for all capsules; preservation of capsules in formalin did not have any significant effect on their weight (Rawlings, unpub. data). The dry weights of the capsular cases were measured as before (see Capsule dry weight, above). Unfortunately, because a number of Ross Islet snails in the 33% food treatment either died (n = 2) or stopped spawning (n = 5), too few capsules were collected for dry weight analysis.

c) Predator-stimulus experiment

Pretreatment conditions: To examine the effect of chemical cues released by capsule-eating predators on the production and morphology of *Nucella emarginata* capsules, in January 1992, I selected 12 reproductively mature female and male F_1 snails from Grappler Inlet, Ross Islet, and Folger Island laboratory populations (n = 36 cages in total). Three cages were each allocated to one of 12 opaque (12 L) buckets (n = 36 cages in total). Cages were assigned in such a way that each bucket contained a F_1 female snail from each source population. Buckets were arranged in pairs, and each pair of buckets was provided with a gravity fed supply of fresh seawater (average rate = 2.0 - 2.5 L/min) from a source tank mounted above. Snails were kept under these conditions for one month prior to exposing snails to treatment conditions.

Treatment conditions: I randomly assigned each bucket to one of three treatment conditions designed to manipulate the exposure of snails to specific capsule-eating predators. Buckets were arranged such that sibling F_1 females were divided equally among treatment conditions. These treatments were: 1) "*Idotea* present" (1 pair of buckets x 2 replicates), 2) "*Hemigrapsus* present " (1 pair of buckets x 2 replicates), and 3) "control" (1 pair of buckets x 2 replicates). In the "*Idotea* present" and "*Hemigrapsus* present" treatments, snails were continuously exposed to water from a source tank containing barnacle-covered rocks and either 8 - 14 *Idotea wosnesenskii* (≈ 5 g, whole wet weight) or 10 - 15 *Hemigrapsus nudus* (≈ 30 - 35 g, whole wet weight). In contrast, snails in the "control" treatment were exposed to water passing through a source tank containing barnacle-covered rocks. Capsule-eating predators were fed 10 *Nucella* egg capsules at 5 d intervals for the first month of this experiment. After this time, due to an increased availability of egg capsules, this number was increased to 20 capsules every 5 d. The number of capsules consumed per source bucket by *Hemigrapsus* and *Idotea* was verified at 15 d intervals by searching for capsular remains. All predators were replaced with freshly-collected ones from the field at monthly intervals.

Capsule wall thickness: Once snails were assigned to their respective treatment conditions, cages were checked every two weeks for capsules. At monthly intervals, all barnacle-covered rocks were replaced with freshly-collected rocks from the field. After three months, one clutch of capsules (> 8 capsules) was collected from all females. Three of these capsules were sectioned from each female snail to determine the effect of treatment conditions on the wall thickness of capsules produced. The remaining capsules were used to compare the susceptibility of capsules to capsule-eating predators (see next section).

Predator-feeding experiments: To determine if capsules produced under the various treatment conditions differed in their vulnerability to predation, I offered capsules produced in the "*Idotea* present", "*Hemigrapsus* present", and "control", treatments to capsule-eating predators. Because previous experiments have shown that isopods, such as *Idotea wosnesenskii* and *Gnorimosphaeroma oregonense,* can discriminate between different morphologies of capsules produced by field populations of *Nucella emarginata* (Rawlings, 1990; 1994; Chapter 3), *Idotea* were used as the capsule-eating predators in this study. Capsules used in these experiments were collected from snails after > 3 months exposure to treatment conditions.

Fifty-four adult *Idotea* were collected from Grappler Inlet in August 1992 and kept in seawater trays with *Fucus gardneri* and *Ulva spp.* until needed. Prior to conducting this experiment, individuals were measured for body length (range: 20.2 - 33.6 mm) and then randomly assigned to one of 18 mesh-paneled cages (three *Idotea* per cage). Each cage was partially immersed in a seawater table and provided with eight egg capsules from each of two treatment conditions (16 capsules in total). Capsules were arranged in two circles of eight capsules, such that capsules from each treatment were interspersed. Capsules were also marked by means of small color coded dots (Lumocolor™ permanent marker) placed at the base of each egg capsule, to ensure that capsules from each treatment could be identified if they became dislodged. Predator preferences were examined for capsules produced in 1) *Idotea* vs. Control treatments (n = 6), 2) *Hemigrapsus* vs. Control treatments (n = 6), and 3) *Idotea* vs. *Hemigrapsus* treatments (n = 6). The number and color code of capsules opened by *Idotea* was recorded daily for each cage. Experiments were terminated when approximately eight out of the 16 egg capsules (i.e., 50%) had been opened per cage.

Results

Intraspecific variation in capsule size, shape, and wall thickness

Capsule size and shape varied substantially among the ten populations sampled in Barkley Sound (Fig. 2-1, Table 2-1). These site-differences were relatively consistent between two separate samples of ten clutches collected from all sites in the summer of 1991 (data not shown, see Chapter 7), and between years (May 1991 and April - June 1992) at four sites that were sampled in 1992 (Fig. 2-2). Capsule size and shape differed substantially between wave-sheltered and wave-exposed sites. Capsules from Grappler Inlet, the most wave-sheltered population, had significantly larger body and chamber lengths and increased chamber volumes relative to other sites. Capsules from wave-exposed shores, however, were stockier in shape, having disproportionately large capsular plugs and chamber widths based on their chamber lengths, compared to wave-sheltered sites. Although the width of the capsule chamber was positively correlated with chamber length within most sites (Table 2-2), capsules from the most wave-exposed shores (e.g., Folger Island, Cape Beale, and Kirby Point) had substantially larger chamber widths per unit chamber length than capsules from less exposed areas (Fig. 2-3; adjusted means: Fig. 2-1). Unlike chamber width, however, the size of the capsular plug did not vary consistently with capsule chamber length within and among populations (data not shown);

allometric relations between capsule plug and chamber length were significant ($\alpha = 0.05$) for only one of ten sites.

The thickness of capsule walls also varied significantly among populations separated along the wave-exposure gradient (Fig. 2-4). This variation was not related to site-differences in capsule size, since wall thickness did not vary with chamber length or capsule body length either within or among populations (Rawlings, 1990). Capsules were typically thickest at the most wave-sheltered or wave-exposed sites and were thinnest at sites intermediate in wave-exposure. This pattern was evident regardless of the index of wave-exposure that was used to rank these sites (i.e., barnacle height, height of vascular plants, or wave amplitude; data not shown) and occurred for both years in which capsules were sectioned (1990, 1991). These results also confirmed site-differences in capsule wall thickness observed in 1988 (Rawlings, 1990; Fig. 2-4: filled symbols). Mean capsule wall thickness did vary from year to year within a site, however. This within-site variation differed significantly among populations over the two years sampled, as indicated by a significant interaction effect between years and sites (see ANOVA, Fig. 2-4).

Variation in capsule wall thickness within sites was not related to the position that capsules were spawned within the intertidal zone (Fig. 2-5). Comparisons of capsule wall thickness between high and low transect lines at Ross Islet and Kirby Point revealed a significant difference in wall thickness between sites, but no effect of transect height (2-way ANOVA: F(site) = 14.45, df = 1, 34, $P < 0.001$; F(height) = 0.00, df = 1, 3, P = 0.97; F(interaction) = 0.48, df = 1, 34, P = 0.49).

The allocation of material to capsular cases also varied substantially among sites (Fig. 2-6). Case dry weight was positively related to capsule wet weight for eight of ten sites sampled in 1991 (Table 2-3). Because the slopes of these site-specific relationships were significantly different, however, average differences in the dry weight of cases for a given wet weight of capsule could not be compared statistically using ANCOVA. Nevertheless, differences among populations in the dry weight of the case (computed for a given wet weight, using the least-squares linear regression equations for each site) appeared to reflect site-differences in capsule wall thickness. For capsules of a constant wet weight (0.025 g), those with heavier case weights were also those with thicker capsule walls (Fig. 2-7). This relationship was also apparent when comparing capsules at wet weights of 0.020 and 0.030 g (see Appendix 1: Fig. A1-1). The same general trends were also apparent in capsules collected from Grappler Inlet, Ross Islet, Kirby Point, and Cape Beale in 1992 (Fig. 2-8, Table 2-3). Because their slopes were not significantly different, these site-specific relationships were compared using ANCOVA. Although thin-walled Ross Islet capsule cases were the lightest per unit wet weight of capsules, they were not significantly different from Grappler and Kirby capsules in this sample. Nevertheless, differences among sites were still in the direction that would be predicted given the increased wall thickness of capsules from Grappler Inlet, Kirby Point, Cape Beale and Folger Island, and the proportionally larger plugs of capsules collected from Kirby Point, Cape Beale and Folger Island, both of which would contribute to the increased weight of the capsule case.

The proportional contribution of the capsule case to the total dry weight of the capsule was also determined for each site over a range of capsule wet weights (Fig. 2-9). Capsule cases contributed substantially to the overall capsule dry weight, although the actual proportion varied with capsule size. For most sites, the proportional allocation of case material decreased with increasing capsule wet weight, accounting for a maximum of 54 - 70% of the dry weight of the whole capsule at a wet weight of 0.02 g.

a) Genetic versus environmental effects on capsule form

The wall thickness of the first clutch of capsules spawned by field-raised snails confined in the laboratory was remarkably similar to capsules produced under field conditions (Figs. 2-4, 2-10). Nevertheless, subsequent capsules produced by each of five snails from both Ross Islet and Grappler laboratory populations varied widely in wall thickness. Over time, however, some females tended to produce thicker-walled capsules under laboratory conditions. The mean thickness of capsule walls produced after 470 d in the laboratory increased significantly from the first clutch of capsules spawned in the laboratory by Ross Islet snails (60.9 ± 1.64 µm vs. 70.0 ± 4.37 µm, n = 5 females, Mann-Whitney U-test: $U = 0.00$, $P < 0.01$), but not for Grappler Inlet snails (85.6 ± 0.88 µm vs. 85.8 ± 3.16 µm, n = 5 females, Mann-Whitney U-test: $U = 10.0$, $P = 0.60$). Interestingly, despite the marked variation in the wall thickness of capsules produced over time, obvious population-differences in the wall thickness of capsules still persisted after one year in the laboratory.

F_1 laboratory-reared snails became reproductively mature at similar times (October/November, 1991) but at different shell lengths for each population: Grappler Inlet: 34.1 ± 0.49 mm (n = 12); Ross Islet: 29.3 ± 0.54 mm (n = 12), and Folger Island: 27.9 ± 1.46 mm (n = 12). Capsules laid by these snails had considerably thicker walls than capsules collected from their respective field populations (Fig. 2-11). To determine if population-differences still persisted in snails born and raised under laboratory conditions, I compared the wall thickness of capsules produced by offspring from each of two families from Grappler, Ross Islet and Folger laboratory populations using a nested ANOVA. Although significant differences were present in the wall thickness of capsules produced by sibling females, no significant differences were found among families and among laboratory populations (Fig. 2-11).

Interestingly, the conditions under which sibling F_1 female snails were raised in the laboratory appeared to have a large effect on the wall thickness of capsules they produced. Sibling females raised for the food ration experiment (see below) produced substantially thinner-walled capsules in pretreatment conditions than snails in the above experiment (Fig. 2-12). The culturing conditions of these snails was identical to those described above (i.e., all snails came from the same rearing cages), except that snails raised for the food ration experiment were kept in higher density rearing cages (15 snails per cage) for six months longer before being transferred to their own mesh-paneled cages. This difference in rearing conditions had a significant effect on the wall thickness of capsules produced by Ross Islet snails. A similar effect of rearing conditions was evident in one of two Grappler Inlet families (Fig. 2-12).

Like capsule wall thickness, capsule size and shape were found to be relatively plastic traits. Overall, larger capsules, with significantly wider chambers per unit length, were produced under laboratory conditions for all three populations (Fig. 2-13; Table 2-4). The relative size of the capsular plug also varied between laboratory and field-spawned capsules. Ross Islet and Grappler Inlet snails both produced capsules with proportionally larger plugs per unit chamber length in the laboratory (Fig. 2-14). Surprisingly, however, the relative plug length did not differ between field- and lab-spawned capsules of Folger Island snails (Fig. 2-14, Table 2-4). Comparisons among capsules produced by laboratory populations indicated that the relationship between chamber width and chamber length did not vary significantly, yet the relationship between capsule plug length and chamber length did differ among Grappler Inlet, Ross Islet, and Folger laboratory-reared snails (Fig. 2-14; Table 2-4).

b) Effect of food ration on capsule morphology

Limiting the exposure of snails to their barnacle prey, *Balanus glandula,* had a substantial effect on the food consumption among treatment groups. Although snails in the 33% ration treatment fed on more barnacles per 10 d period than snails in the 100% ration treatment, when the consumption of food was estimated for the full 30 d cycle, snails in the 100% ration treatment consumed 2.05, 2.08, and 2.09 times as much dry weight of barnacle flesh as snails on the 33% ration, for Grappler, Ross Islet, and Folger laboratory populations, respectively (Fig. 2-15).

Despite these differences in food consumption, ration treatment had little effect on snail shell growth over the period. This likely resulted from the fact that all snails were reproductively mature prior to the start of treatment conditions, and hence, grew little during the subsequent treatment period (Fig. 2-16; Table 2-5). Significant differences were present in shell-length among laboratory populations, however, thus reflecting genetic differences in size at reproductive maturity.

The morphology of capsules varied somewhat among ration treatments. Capsules produced by snails in the 100% treatment group had larger capsule body lengths and chamber lengths than capsules produced in the lower food ration groups (Table 2-5), but these differences were not quite significant at $\alpha = 0.05$ (Table 2-6). This effect of food ration did appear to differ among populations, with Grappler Inlet snails showing a much larger response to changes in food ration than snails from the other two sites (Table 2-5). No differences in plug length, chamber width or overall chamber volume were evident among treatment groups. Significant differences in capsule size and volume were still maintained among Grappler, Ross and Folger laboratory populations, however (Tables 2-5, 2-6).

In contrast to the effect on capsule size, ration treatment had a substantial effect on the packaging of embryos with egg capsules. Snails in the 100% food ration treatment enclosed significantly more embryos per capsule, and more embryos per unit capsule volume compared to capsules produced in 33% and 66% ration treatments (Tables 2-5, 2-6). Despite this, food ration had no effect on the thickness of capsule walls (Fig. 2-17; Tables 2-5, 2-6). Capsule wall thickness increased significantly in all treatment groups during the course of the experiment (5.4 µm, 6.4 µm, and 8.8 µm for Grappler Inlet, Ross Islet, and Folger Island snails, respectively), but treatment condition had no effect. Likewise, regressions of the dry weight of the capsular case versus wet weight of whole capsules collected from 33% and 100% ration treatments also did not reveal any significant difference in the allocation of material to capsular cases per unit wet weight of capsule for Grappler and Folger laboratory populations (Table 2-7; Appendix 1: Fig A1-2). Grappler snails on 100% ration, however, did produce heavier whole capsules than snails on 33% ration (Table 2-7).

Differences in food ration treatment had a significant effect on the number of capsules produced by snails over the one-year treatment period (Fig. 2-18). Snails in the 33% ration treatment spawned significantly fewer capsules relative to snails in the 66% and 100% treatment groups. Differences in spawning among treatments were most evident in the fall and winter months during a period when snails in the field and laboratory typically decrease their capsule production (Palmer, 1994; Rawlings, pers. obs.). Populations also differed substantially in the number of capsules spawned on the same food ration, and in the seasonality of capsule production. Snails from Folger Island exhibited only a weak seasonal change in spawning rate compared to Grappler Inlet and Ross Islet snails.

c) Effect of predator-stimulus on capsule wall thickness

Crab and isopod predators fed readily on *Nucella emarginata* capsules provided to them as a source of food during this experiment. Few capsules remained intact within the predator-holding buckets at the end of 15 d. The exposure of snails to water borne cues released by capsule-eating predators, however, had little effect on number of capsules spawned over the three month treatment period. Capsule production continued unabated in all treatment groups regardless of the predator stimulus (Fig. 2-19). Likewise, the wall thickness of capsules produced by snails was unaffected by treatment stimulus (Fig. 2-20).

Predator-feeding experiments confirmed that there were no differences in the protective quality of capsules produced by female snails exposed to the different treatment conditions. The predatory isopods, *Idotea wosnesenskii,* did not exhibit any preference for capsules spawned under any treatment condition (Fig. 2-21).

Discussion

Intraspecific variation in capsule form

Within the marine environment, the intensity of environmental stresses varies greatly along intertidal shores exposed to differential wave action. Wave-exposure gradients thus offer a convenient starting point for intraspecific comparisons of life-history traits. Increased wave exposure is associated with 1) decreased predation intensity, as wave-action can tend to disrupt predator foraging activities (e.g., Kitching et al., 1959; Menge, 1978a; 1978b; Robles, 1987), and 2) reduced desiccation stress, resulting from increased wave-spray on wave-exposed shores (Dayton, 1971; Menge, 1978a). Wave action itself, however, can also impose severe physical stresses upon intertidal organisms due to dislodgement (Denny, 1985; Denny et al., 1985, Etter, 1988) and damage imposed by water-born debris (Dayton, 1971; Shanks and Wright, 1986). These effects can have significant consequences for the growth rate (e.g., Janson, 1982; Brown and Quinn, 1988; Etter, 1989), shape (e.g., Crothers, 1984; Janson, 1983; Etter, 1988; 1989; Gibbs, 1993; Palmer et al., unpub. data), and size (e.g., Ebert, 1982; Denny et al., 1985; Etter, 1988; 1989; Palmer, et al., unpub. data) of many intertidal organisms.

Like many benthic marine organisms, the morphology of *Nucella emarginata* capsules varied extensively among populations separated along a gradient of wave-exposure (Table

2-8). Site-site variation was evident not only in capsule size and shape, but also in capsule wall thickness and the relative weight of the capsular case. Although these site-differences in capsule form were relatively consistent between years, (see Fig. 2-1, 2-2, 2-4), variation among populations was not a direct function of wave-exposure, per se. In fact, capsule form differed little among populations from shores of low to moderate wave-action (Table 2-8); instead, capsule form differed most at the wave-sheltered (i.e., no wave action: Grappler Inlet) and wave-exposed sites (Kirby Point, Grappler Inlet, Folger Island) (see Table 2-8).

The fact that wave action can exert so many direct and indirect effects on intertidal organisms makes deducing the cause(s) of differences in life-history traits, once found, particularly difficult. This is especially true for intraspecific comparisons of capsule form among neogastropod molluscs. Because the final form of *Nucella* egg capsules is molded by the ventral pedal gland located in the mesopodium of the foot, interpopulation variation in some components of capsule form may simply be a byproduct of ecophenotypic differences in snail morphology. Shell length, shape, aperture length, and foot size of adult snails can vary extensively among populations of *Nucella* exposed to different flow regimes (Crothers, 1984; Etter, 1988; Palmer, 1992; Gibbs, 1993; Palmer et al., unpub. data). Hence, even though interpopulation differences in capsule form can have important consequences for the packaging of embryos within capsules (see Spight and Emlen, 1976; Gallardo, 1979; Pechenik et al., 1984), the proportion of reproductive energy devoted towards capsule versus embryo production (Perron, 1981; Perron and Corpuz, 1982), and the survival of encapsulated embryos within these structures (Perron, 1981; Rawlings, 1990; Chapter 3, 4, 5, 7), such consequences do not ensure that this variation is adaptive.

How can one interpret the adaptive significance of variation in capsule form? Generally several lines of evidence are required to support an adaptive explanation for variation in any given trait (see reviews in Stearns, 1989; Thompson, 1991; Travis, 1994). First, variation in the expression of a trait must have some heritable basis. Phenotypic differences in capsule form among populations can arise via genetic variation, direct environmental variation, or more complex genotype-environment interactions (e.g., Via and Lande, 1985; Stearns and Koella, 1986). The evolutionary significance of variation in reproductive patterns and spawn morphology, therefore, depends on the extent to which some portion of this variation is heritable. If heritable differences are observed among populations, and if gene flow is restricted, then interpopulation differences may arise via natural selection. Thus, we must know if site-differences in capsule form are environmentally or genetically based. Second, although natural selection requires a genetic component to phenotypic variation, phenotypic plasticity in capsule form does not necessarily rule out potential adaptive explanations for among-population differences in these traits. Many phenotypically plastic life-history traits have an underlying genetic basis (e.g., genotype-environment interactions), and where genetic variation for plasticity exists, a population with a different mean phenotypic plasticity may evolve (Stearns, 1989; Thompson, 1991; Travis, 1994). Hence, the response of phenotypically-plastic traits to different environmental conditions must also be assessed to determine if these traits vary between environments in an adaptive manner. Third, to interpret the adaptive significance of various capsule traits, one must also demonstrate the fitness-related effects associated with differences in capsule form.

Genetic versus environmental effects on capsule form

Some variation in the reproductive characteristics of *N. emarginata* populations did appear to have an underlying genetic basis (Table 2-9). Female size at reproductive maturity, for instance, differed significantly among populations of laboratory-reared snails (Fig. 2-16). This difference was also reflected in the absolute size of capsules spawned by laboratory populations of snails: capsules from Grappler Inlet snails had significantly larger body and chamber lengths compared to those from Ross Islet and Folger Island (Tables 2-5, 2-6). Capsule size, however, was also affected substantially by changes in environmental conditions (Figs. 2-13, 2-14; Tables 2-5, 2-6). In addition, laboratory-raised snails also exhibited remarkable differences among populations in their seasonality of spawning (Fig. 2-17), as observed in other populations of laboratory-raised *N. emarginata* (Palmer, 1994). For example, Grappler Inlet and Ross Islet snails exhibited a substantial decline in capsule production in fall and winter months, whereas capsule production by Folger Island snails continued relatively unabated throughout the year. Although capsule production has not been monitored year-round at field populations, anecdotal observations, based on capsule availability, suggest that the seasonality of spawning may also vary among these sites in the field (Rawlings, pers. obs.).

Differences in the morphology of egg capsules among populations appeared to be largely environmentally-induced (Table 2-9). Capsule size increased substantially in the laboratory relative to field-spawned capsules (Figs. 2-13, 2-14), thus reflecting the increased size of reproductively mature snails within this common environment. Relative chamber width also increased in lab-laid relative to field-spawned capsules, as did relative plug length for Ross Islet and Grappler Inlet populations. Hence, capsules produced in the laboratory tended towards a common form, with longer, wider chambers, and proportionally larger plugs, than capsules spawned under field conditions.

Some attributes of capsule shape did appear to have an underlying genetic basis, however. The relation between plug length and chamber length differed significantly among sites, even under laboratory conditions (see Fig. 2-14). Also, unlike capsules from Grappler Inlet and Ross Islet, the relative plug length of Folger Island capsules did not differ between laboratory and field conditions (Fig. 2-14; Table 2-4). Thus, because these responses were population-specific, genetic effects must be responsible for some interpopulation variation in the plug length of *N. emarginata* capsules.

Differences in the wall thickness of capsules among populations of *N. emarginata* also appeared to be largely environmentally-induced. Snails born and raised within the laboratory all produced significantly thicker-walled capsules than under field conditions, regardless of the source of the laboratory population (Fig. 2-11; Table 2-9). Even small differences in the rearing conditions of snails within the laboratory appeared to have a significant effect on the thickness of capsules spawned, at least for one population of snails (Fig. 2-12). Despite this, however, the mean wall thickness of capsules spawned by individual laboratory-raised F_1 females (pooled for females in all pretreatment conditions) differed significantly among populations (means ± SE of : 96.4 ± 1.35 µm, n = 32; 86.3 ± 3.16 µm, n = 36, and 109.3 ± 2.03 µm, n = 21 females, for Grappler Inlet, Ross Islet, and Folger Island snails, respectively; ANOVA, $F = 18.86$; $P < 0.001$; df = 2, 86), with Ross Islet snails producing thinnest capsule walls. Likewise, the magnitude of change in capsule wall thickness between field and laboratory environments also differed significantly, with an average change in wall thickness of 17.4, 33.7 and 23.0 µm, for Grappler Inlet, Ross Islet, and Folger Island snails, respectively. Although this difference in response could simply reflect the relative difference between laboratory and field conditions for each population, this seems unlikely since Ross Islet capsules showed the greatest change in wall thickness between these two environments. *A priori* Folger Island capsules were expected to exhibit the greatest response, given the extreme difference between conditions at this wave-exposed site and the benign laboratory environment. Thus, although capsule wall thickness is clearly a phenotypically plastic trait, genetic differences exist among populations of *Nucella emarginata* in the plasticity of this trait. Direct confirmation, however, will require a more rigorous analysis of the variation in capsule form among environments (i.e., genotype versus environment interactions; see Via and Lande, 1985; Stearns and Koella, 1986; Via, 1993).

Induced changes in capsule form

Nucella do exhibit adaptive induced responses to cues in their environment. Among-population differences in the shell morphology of *Nucella* species, for instance, once assumed to reflect genetic differences, at least partly result from adaptive induced responses to crustacean predators and the scent of damaged conspecifics (Appleton and Palmer, 1988; Palmer, 1990b). Likewise, changes in relative foot size among wave-sheltered and wave-exposed populations of *Nucella lapillus* may also reflect adaptive developmental changes in growth due to specific environmental cues (e.g., Etter, 1988; Gibbs, 1993). Despite this, however, exposure of spawning females to chemical cues released by capsule-eating predators had very little effect on the reproductive output or spawn morphology of *N. emarginata* individuals. Contrary to expectation, an increased risk of predation did not result in thicker-walled capsules or capsules more resistant to isopod or crab predators (Fig. 2-20, 2-21), even though both can be an important source of embryo mortality in the field, and isopods preferentially open thin-walled capsules when given a choice (Rawlings, 1990; Chapter 3). Nevertheless, under the conditions examined here, interpopulation differences in the protective quality of capsule walls did not appear to be induced responses to isopod or crab predators.

Somewhat reassuringly, however, changes in food ration also failed to produce differences in the wall thickness of capsules spawned by *Nucella emarginata* (Fig. 2-18). Hence, site-differences in the protective quality of capsules were not the result of proximate differences in food abundance among field populations. Nevertheless, food ration did have significant effects on other reproductive characteristics. Laboratory populations of *Nucella emarginata* reared on 100% ration levels spawned significantly more capsules that were larger in size and contained more embryos per unit chamber volume compared to capsules produced by snails on lower food rations, hence, again suggesting an environmental influence on the expression of these traits (Table 2-9). Interestingly, populations differed in their response to food ration, with Grappler snails exhibiting substantially greater responses than snails from Ross Islet and Folger Island. These differences in response among populations were not significant, however (Table 2-5).

Although the reproductive characteristics of other gastropods are also affected substantially by changes in food availability (e.g., *Nassarius pauperatus*; McKillup and Butler, 1979; *Nucella emarginata* and *N. lamellosa*, Spight and Emlen, 1976), some species differ markedly in their response compared to *Nucella emarginata*. Populations of the mud snail *Nassarius pauperatus* with low food availability, for instance, spawned more eggs and more egg capsules per female, but fewer eggs per individual capsule than populations with abundant food (McKillup and Butler, 1979). Further studies on the effects of food availability on reproductive characteristics of marine gastropods, therefore, may prove fruitful in determining a) how snail populations are take to take advantage of seasonal food supplies in temporally changing environments (see Spight and Emlen, 1976) and b) if these responses differ among populations.

Adaptive significance of variation in capsule form.

Are adaptive explanations for variation among populations in capsule morphology possible given the extensive plasticity in capsule form? Plasticity in several aspects of capsule morphology does not necessarily rule out adaptive explanations for among-population differences in these traits, since differences in the plasticity of life-history traits often have a genetic basis (see Stearns, 1989; Thompson, 1991; Travis, 1994). Likewise, the lack of an induced adaptive response to specific environmental conditions is not in itself very informative, especially given the endless number of variables that can potentially affect capsule morphology. Clearly, the only way to further understand the genetic basis of variation in capsule form is to undertake a more rigorous examination of the reproductive responses of representative populations of *N. emarginata* to *two* or more different environmental conditions (see Via and Lande, 1985; Via, 1993). Significant genotype by environment interactions resulting from such experiments would allow adaptive explanations for interpopulation differences in capsule form to be proposed. Although common garden experiments, such as those conducted in the present study, are a useful exercise in generating a crude measure of the relative influence of genetic versus environmental effects on variation in capsule form, they do not provide conclusive information on genetic versus environmental interactions, unless more than one common garden is examined (see Table 2-9). Nevertheless, since some differences among populations of *N. emarginata* were observed in capsule size, relative plug length, and mean capsule wall thickness under common laboratory conditions, variation in these traits may be heritable to some degree.

The adaptive significance of differences in capsule form can also be tested by direct comparisons of fitness-related effects of specific capsule traits. Subtle differences in capsule form can have important implications for the development and survival of encapsulated embryos. The thickness of capsule walls, for instance, is a measure of the investment in embryo protection (Perron, 1981; Perron and Corpuz, 1982; Etter, 1989; Rawlings, 1990). Within the genus *Conus*, thicker-walled capsules resisted puncturing better than thinner walled capsules, and hence, are more likely to resist grazing by capsule-eating predators (Perron, 1981; Perron and Corpuz, 1982). Furthermore, thick-walled capsules of *Nucella emarginata* are significantly more resistant to isopod predators (Rawlings, 1990; Chapter 3), absorb a higher percentage of incident UV-B radiation

(Chapter 5), and, under certain environmental conditions, may also reduce the rate of water loss from the capsule chamber, relative to thinner walled capsules (Chapter 4).

Differences in capsule form can incur specific costs, however. Thicker capsule walls may result in a substantial energetic expense (Fig. 2-7). Thicker protective barriers may also constrain the rate of development of encapsulated embryos by retarding the movement of oxygen and metabolic wastes across these structures (Strathmann and Chaffee, 1984; Chapter 7). Capsule form may thus reflect a tradeoff between the costs of enclosing eggs within thick-walled capsules and the benefits of increased embryonic protection. Clearly, therefore, such cost - benefit studies can be of tremendous use in helping to interpret potential adaptive functions associated with specific capsule traits.

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Table 2-1. ANOVA tables for comparisons of capsule size and shape among ten populations of *Nucella emarginata* in 1991 and four populations in 1992. Mean lengths, volumes, shape indices, and sample sizes for each site are presented in Figures 2-1 and 2-2, as are the results of *a posteriori* multicomparison tests.

	1991				1992			
Capsule Dimension	Mean Square	df	F	P	Mean Square	df	F	P
Capsule Body Length	Sites: 4.232	9	21.543	<0.001	Sites: 39.688	3	129.107	<0.001
	Error: 0.196	89			Error: 0.307	197		
Capsule Chamber	Sites: 2.892	9	17.912	<0.001	Sites: 31.997	3	155.740	<0.001
	Error: 0.161	89			Error: 0.205	197		
Capsule Volume	Sites: 315.098	9	22.974	<0.001	Sites: 1442.769	3	90.936	<0.001
	Error: 13.715	89			Error: 15.866	197		
Plug/Chamber Length	Sites: 93.188	9	15.536	<0.001	Sites: 471.069	3	46.899	<0.001
	Error: 5.998	89			Error: 10.044	197		

Table 2-2. Log-log regression equations describing associations between chamber lengths (X, in mm) and chamber widths (Y, in mm) for *Nucella emarginata* egg capsules collected from 10 different sites in Barkley Sound. N - sample size; slope (± SE) - slope of ordinary least squares linear regression; r^2 - coefficient of determination; P - probability that OLS slope deviates from zero; intercept (± SE) - intercept of OLS regression; RMA slope - slope of the reduced major axis regression equation. Regression equations were compared among sites using ANCOVA. Adjusted means are given in Fig. 2-1.

SITE†	N	r^2	Slope (± SE)	P	Intercept (± SE)	RMA Slope
Grappler	20	0.316	0.313 (0.1083)	0.01	0.281 (0.0807)	0.557
Ross Islet	20	0.044	0.146 (0.1596)	0.373	0.357 (0.1043)	....
Kelp Bay	20	0.626	0.891 (0.1623)	<0.001	-0.124 (0.0990)	1.126
Dixon Island	20	0.531	0.564 (0.1250)	<0.001	0.090 (0.0777)	0.774
Self Point	20	0.175	0.523 (0.2674)	0.066	0.124 (0.1523)	1.250
Wizard Rock	20	0.110	0.215 (0.1440)	0.152	0.302 (0.0893)	....
Voss Point	20	0.254	0.483 (0.1948)	0.023	0.158 (0.1137)	0.958
Kirby Point	20	0.361	0.484 (0.1516)	0.005	0.170 (0.0953)	0.806
Cape Beale	19	0.315	0.404 (0.1445)	0.010	0.263 (0.0927)	0.720
Folger Island	21	0.024	0.148 (0.2148)	0.498	0.388 (0.1279)	....

† ANCOVA: Significance value for slopes: $P = 0.150$; adjusted means: $P < 0.001$. See Fig. 2-1 for adjusted means for each site.

Table 2-3. Associations between a) Log case dry weight (Y-axis, in g) *vs.* Log whole wet weight (X-axis, in g), and b) Log whole dry weight (Y-axis, in g) *vs.* Log whole wet weight (X-axis, in g) for *N. emarginata* capsules collected from 10 different sites in Barkley Sound in 1991, and four different sites in 1992. Column headings are defined in Table 2-2.

SITE	Year	N	r^2	Slope (±SE)	P	Intercept (±SE)	RMA
a) Log (Case dry weight, Y) versus Log (Whole wet weight, X)							
Grappler Inlet	1991	10	0.642	0.6576 (0.17359)	<0.01	-1.6238 (0.26808)	0.821
	1992	25	0.672	0.6996 (0.10199)	<0.001	-1.5941 (0.14188)	0.854
Ross Islet	1991	22	0.312	0.4367 (0.14507)	<0.01	-2.0909 (0.23966)	0.782
	1992	25	0.689	0.6376 (0.08926)	<0.001	-1.7043 (0.14811)	0.769
Kelp Bay	1991	10	0.194	0.6041 (0.43483)	0.202	-1.7751 (0.74512)	....
Dixon Island	1991	13	0.719	0.6957 (0.13113)	<0.001	-1.6199 (0.22074)	0.821
Self Point	1991	14	0.520	0.6108 (0.16949)	<0.01	-1.7866 (0.29943)	0.847
Wizard Rock	1991	13	0.207	0.9074 (0.53473)	0.118	-1.2894 (0.89025)	....
Voss Point	1991	13	0.716	0.7607 (0.14460)	<0.001	-1.4786 (0.24164)	0.899
Kirby Point	1991	26	0.746	1.1999 (0.14279)	<0.001	-0.7431 (0.23491)	1.389
	1992	25	0.861	0.8094 (0.06793)	<0.001	-1.4032 (0.11221)	0.872
Cape Beale	1991	13	0.931	1.1054 (0.09108)	<0.001	-0.8597 (0.13600)	1.145
	1992	25	0.669	0.7687 (0.11269)	<0.001	-1.4037 (0.17622)	0.940
Folger Island	1991	12	0.766	1.0624 (0.18557)	<0.001	-0.8932 (0.30002)	1.213

Table 2-3(b)

SITE	Year	N	r^2	Slope (SE)	P	Intercept (SE)	RMA
b) Log (Whole dry weight, Y) versus Log (Whole wet weight, X)							
Grappler Inlet	1991	10	0.853	0.6822 (0.09994)	<0.001	-1.3289 (0.15058)	0.738
	1992	25	0.841	0.9263 (0.08404)	<0.001	-0.9984 (0.11619)	1.010
Ross Islet	1991	26	0.866	0.9383 (0.07524)	<0.001	-0.9706 (0.12421)	0.008
	1992	25	0.930	0.8072 (0.04632)	<0.001	-1.1436 (0.07648)	0.837
Kelp Bay	1991	10	0.924	0.9433 (0.09573)	<0.001	-0.9481 (0.16367)	0.981
Dixon Island	1991	14	0.937	0.9122 (0.06852)	<0.001	-1.0327 (0.11741)	0.942
Self Point	1991	14	0.865	0.8273 (0.09448)	<0.001	-1.1691 (0.16692)	0.889
Wizard Rock	1991	14	0.753	0.8783 (0.14502)	<0.001	-1.0649 (0.24502)	1.012
Voss Point	1991	13	0.744	0.5895 (0.10416)	<0.001	-1.5122 (0.17778)	0.684
Kirby Point	1991	24	0.936	1.0199 (0.05682)	<0.001	-0.7885 (0.09343)	1.054
	1992	25	0.958	0.9570 (0.04163)	<0.001	-0.8852 (0.06856)	0.978
Cape Beale	1991	13	0.896	1.3745 (0.14110)	<0.001	-0.2448 (0.21057)	1.453
	1992	25	0.891	0.9730 (0.07095)	<0.001	-0.8497 (0.11359)	1.031
Folger Island	1991	12	0.773	1.1201 (0.19172)	<0.001	-0.6253 (0.30785)	1.274

Table 2-4. Associations between a) Log chamber width (Y-axis, in mm) versus Log chamber length (X-axis, in mm) and b) Log plug length (Y-axis, in mm) versus Log chamber length (X-axis, in mm), for capsules spawned by laboratory F1 snails versus capsules spawned in the field for three populations of *Nucella emarginata*. Column headings are described in Table 2-2. See Figures 2-13 and 2-14 for scatterplots.

			Regression Equation:				
SITE	N	r^2	Slope (SE)	P	Intercept (SE)	RMA Slope	Adjusted Mean (±SE)
a) Log (Capsule chamber width, Y) versus Log (Capsule chamber length, X)							
Grappler Inlet:							
Lab	23	0.016	0.102 (0.1737)	0.564	0.485 (0.1397)	....	0.555 (0.0076)
Field	38	0.264	0.297 (0.0825)	0.001	0.291 (0.0609)	0.578	0.516 (0.0057)
		Significance:†	0.321				< 0.001
Ross Islet:							
Lab	23	0.039	0.137 (0.1470)	0.363	0.436 (0.1076)	0.688	0.520 (0.0079)
Field	40	0.230	0.473 (0.1405)	0.002	0.143 (0.940)	0.987	0.467 (0.0056)
		Significance:†	0.163				< 0.001
Folger Island:							
Lab	20	0.207	0.319 (0.1471)	0.044	0.316 (0.1034)	0.701	0.528 (0.0100)
Field	21	0.026	0.151 (0.2145)	0.489	0.387 (0.1277)	....	0.487 (0.0097)
		Significance:†	0.561				0.024
ANCOVA (among laboratory populations):							
		Significance: †	0.654				0.119

† Significance levels for homogeneity of slopes and adjusted means using ANCOVA

			Regression Equation:				
SITE	N	r^2	Slope (± SE)	P	Intercept (±SE)	RMA Slope	Adjusted Mean (±SE)
b) Log (Capsule plug, Y) versus Log (Capsule chamber, X)							
Grappler Inlet:							
Lab	23	0.024	-0.166 (0.2319)	0.482	0.437 (0.1865)	....	0.303 (0.0116)
Field	28	0.012	0.091 (0.1610)	0.577	0.126 (0.1187)	....	0.193 (0.0103)
		Significance:†	0.407				< 0.001
Ross Islet:							
Lab	23	0.242	0.894 (0.3458)	0.017	-0.408 (0.2532)	1.817	0.215 (0.0125)
Field	30	0.412	1.041 (0.2351)	<0.001	-0.578 (0.1592)	1.622	0.149 (0.0106)
		Significance:†	0.725				<0.001
Folger Island:							
Lab	21	0.435	1.188 (0.3105)	0.001	-0.571 (0.2189)	1.801	0.231 (0.0133)
Field	10	0.097	0.396 (0.4285)	0.382	-0.027 (0.2527)	....	0.281 (0.02420
		Significance:†	0.168				0.147
ANCOVA (among laboratory populations):							
		Significance:‡	0.003				

---

† Significance levels for homogeneity of slopes and adjusted means using ANCOVA

‡ Since slopes were heterogeneous, adjusted means were not compared.

Table 2-5. Summary of the mean (± SE) snail shell lengths (in mm), capsule dimensions (in mm), capsule volume (in µl), number of embryos per capsule, and number of capsules spawned, for snails from Grappler Inlet, Ross Islet and Folger Island laboratory populations raised for > 6 months on one of three diet ration treatments: 33%, 66% and 100% ration. Sample sizes (in brackets) refer to the number of snails sampled from each treatment condition. Up to six capsules were sampled per female. Asterisks indicate significant differences among ration levels (see Table 2-6 for ANOVA results).

a) GRAPPLER INLET:

RATION TREATMENT:	33% (9)	66% (8)	100% (8)
Snail Shell length			
Start	32.0 ± 0.48	31.7 ± 0.54	32.2 ± 0.29
End	32.2 ± 0.47	32.1 ± 0.52	32.6 ± 0.33
Capsule Body Length	7.87 ± 0.217	7.97 ± 0.197	8.56 ± 0.183*
Plug Length	1.89 ± 0.043	1.81 ± 0.053	1.92 ± 0.052
Chamber Length	5.97 ± 0.199	6.16 ± 0.179	6.67 ± 0.179*
Chamber Width	3.69 ± 0.072	3.70 ± 0.084	3.74 ± 0.067
Chamber Volume	42.65 ± 2.186	44.54 ± 2.847	48.85 ± 0.168
# Embryos / Capsule	23.2 ± 2.79	25.7 ± 1.70	34.9 ± 2.61***
# Embryos / Unit Vol.	0.54 ± 0.051	0.59 ± 0.054	0.72 ± 0.057**
Total # Capsules Produced†	**90.6 ± 3.45** **(9)**	**108.1 ± 5.15** **(9)**	**121.9 ± 2.88***** **(9)**

† Note: sample sizes differ from previous measures.

* P ≈ 0.05; **P < 0.01; ***P < 0.001

b) ROSS ISLET:

RATION TREATMENT	33% (3)	66% (8)	100% (9)
Shell Length:			
Start	28.4 ± 0.49	28.4 ± 0.39	27.9 ± 0.40
End	28.6 ± 0.46	28.9 ± 0.39	28.5 ± 0.30
Capsule Body Length	6.51 ± 0.618	6.65 ± 0.110	6.75 ± 0.150*
Capsule Plug	1.52 ± 0.128	1.57 ± 0.04	1.59 ± 0.061
Chamber Length	4.99 ± 0.509	5.09 ± 0.113	5.16 ± 0.107 *
Chamber Width	3.37 ± 0.202	3.39 ± 0.044	3.44 ± 0.060
Chamber Volume	30.59 ± 6.195	30.69 ± 0.816	31.96 ± 1.205
# Embryos / Capsule	19.1 ± 3.05	16.6 ± 1.82	23.8 ± 2.46***
# Embryos / Unit Vol.	0.64 ± 0.035	0.54 ± 0.061	0.76 ± 0.089**
Total # Capsules Produced†	**58.5 ± 6.43 (7)**	**103.3 ± 7.01 (8)**	**102.00 ± 9.10*** (9)**

c) FOLGER ISLAND:

RATION TREATMENT	33% (9)	66% (8)	100% (8)
Shell Length:			
Start	25.7 ± 0.47	25.4 ± 0.51	25.7 ± 0.46
End	26.1 ± 0.49	26.1 ± 0.49	26.3 ± 0.049
Capsule Body Length	6.43 ± 0.210	6.52 ± 0.188	6.67 ± 0.221*
Plug Length	1.72 ± 0.075	1.71 ± 0.062	1.76 ± 0.083
Chamber Length	4.73 ± 0.149	4.84 ± 0.158	4.91 ± 0.156*
Chamber Width	3.56 ± 0.078	3.45 ± 0.087	3.45 ± 0.051
Chamber Volume	31.72 ± 2.154	30.44 ± 2.061	30.78 ± 1.588
# Embryos / Capsule	14.5 ± 1.64	15.4 ± 0.90	18.3 ± 1.01***
# Embryos / Unit Vol.	0.47 ± 0.046	0.52 ± 0.036	0.60 ± 0.036**
Total # Capsules Produced†	**62.9 ± 2.37 (9)**	**85.9 ± 8.53 (9)**	**96.8 ± 6.74*** (9)**

Table 2-6. Results from Model I ANOVA for comparisons of capsule dimensions, capsule wall thickness, the packaging of embryos per capsule, and the total number of capsules spawned by snails from three laboratory populations of *Nucella emarginata* (Grappler Inlet, Ross Islet, and Folger Island) reared for > 6-months on three diet ration treatments: 33% ration, 66% ration, and 100% ration. Also shown are the results of *a posteriori* Tukey Multiple Comparison tests (α = 0.05, unless indicated otherwise). For actual data see Table 2-5.

a) Capsule Body Length:

Source	df	Mean .Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	0.885	2.652	0.078	33%, 66%, < 100%† 33% < 66%, 100%
Study Population	2	19.742	59.165	< 0.001	FG, RI, < GR
Interaction	4	0.206	0.616	0.653	
Error	63	0.33			

†(α = 0.08)

b) Capsule Plug Length:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	0.063	1.400	0.254	
Study Population	2	0.534	11.909	<0.001	RI < FG < GR
Interaction	4	0.017	0.372	0.828	
Error	63	0.045			

c) Capsule Chamber Length:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	0.642	2.733	0.073	33%, 66%, < 100%† 33% < 66%, 100%
Study Population	2	14.680	62.531	<0.001	FG, RI, < GR
Interaction	4	0.193	0.820	0.517	
Error	63	0.235			

†(α = 0.10)

d) Capsule Chamber Width

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	0.030	0.523	0.595	
Study Population	2	0.421	7.339	<0.001	RI, FG, < GR
Interaction	4	0.023	0.400	0.808	
Error	63	0.057			

e) Capsule Volume:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	34.199	0.916	0.405	
Study Population	2	1666.368	44.636	<0.001	FG, RI, < GR
Interaction	4	29.278	0.784	0.540	
Error	63	37.332			

f) Embryo Number per Capsule:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	338.722	9.567	<0.001	33%, 66%, < 100%
Study Population	2	948.742	26.796	<0.001	FG, RI, < GR
Interaction	4	44.763	1.264	0.293	
Error	63	35.41			

g) Embryo Number per Unit Capsule Volume:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	0.168	6.193	0.004	33%, 66%, < 100%
Study Population	2	0.085	3.126	0.051	FG, GR, < RI FG, < GR, RI
Interaction	4	0.014	0.509	0.729	
Error	63	0.027			

h) Capsule Production:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Food Ration	2	9995.179	30.344	<0.001	33%, < 66%, 100%
Study Population	2	4719.528	14.328	<0.001	FG, GR, < RI
Interaction	4	554.167	1.682	0.164	FG, < GR, RI,
Error	69	329.399			

i) Capsule Wall Thickness:

Source	df	Mean Square	F-ratio	P	Tukey M.C.T.
Date‡	1	706.580	10.708	0.002	Pre-treat.<Post-treat.
Food Ration	1	2.281	0.035	0.853	
Study Population	2	3837.482	58.159	<0.001	RI, < GR, < FG
Date x Population	2	15.683	0.238	0.789	
Date x Ration	1	71.941	1.090	0.302	
Pop. x Ration	2	18.843	0.286	0.753	
Date x Pop. x Ration	2	31.468	0.466	0.624	
Error	48	65.983			

‡ Date refers to comparisons between Pre-treatment *vs.* Post-treatment capsules

Table 2-7. Regression equations for associations between a) Log case dry weight (Y-axis, in g) versus Log whole wet weight (X-axis, in g) and b) Log whole dry weight (Y-axis in g) versus Log whole wet weight (X-axis, in g), for capsules produced by Grappler Inlet and Folger Island snails in 33% versus 100% diet ration treatments. Also shown are the ANCOVA results comparing the effect of diet ration on the dry weight of capsular cases and whole capsules using capsule wet weight as the covariate (see Fig. A2-2 (Appendix) for a scatterplot of these data).

					Regression Equation:			
RATION	X-variable	Y-variable	r^2	n	Slope (±SE)	P	Elevation (±SE)	Adjusted Means (±SE)
Grappler Inlet:								
33%	Wet weight	Dry case	0.638	9	0.772 (0.2200)	<0.01	-1.338 (0.2818)	-2.296 (0.0115)
100%	Wet weight	Dry case	0.544	9	0.745 (0.2576)	0.023	-1.355 (0.3091)	-2.279 (0.0115)
			Significance:†		0.937			0.382
Folger Island:								
33%	Wet weight	Dry case	0.854	9	1.163 (0.1819)	<0.001	-0.795 (0.2539)	-2.410 (0.0128)
100%	Wet weight	Dry case	0.732	9	1.026 (0.2344)	0.003	-0.993 (0.3239)	-2.417 (0.0128)
			Significance:†		0.670			0.681
Grappler Inlet:								
33%	Wet weight	Dry whole	0.939	9	0.984 (0.0943)	<0.001	-0.850 (0.1187)	-2.053 (0.0073)
100%	Wet weight	Dry whole	0.873	9	0.774 (0.1116)	<0.001	-1.081 (0.1327)	-2.030 (0.0073)
			Significance:†		0.189			0.052
Folger Island:								
33%	Wet weight	Dry whole	0.774	9	1.029 (0.2102)	0.002	-0.716 (0.2861)	-2.133 (0.0154)
100%	Wet weight	Dry whole	0.716	9	1.226 (0.2914)	0.004	-0.449 (0.4054)	-2.135 (0.0154)
			Significance:†		0.598			0.899

† Significance levels for homogeneity of slopes and adjusted means using ANCOVA.

Table 2-8. Qualitative values of capsule attributes among ten populations of *Nucella emarginata* separated along a gradient of wave-exposure in Barkley Sound. The actual data on which this table is based can be found in the figures and tables indicated in the right-hand column.

Trait	EXPOSURE TO WAVE ACTION None (GR)	Low (RI, KB)	Moderate (DX, SP, WZ, VP)	High (KP, CB, FG)	Figure/Table
Chamber Length	High	Low	Low	Low	Fig. 2-1, 2-2
Relative Chamber Width†	Med	Low	Low	High	Fig. 2-1, 2-2, 2-3
Relative Plug Length†	Low	Low	Low	High	Fig. 2-1, 2- 2
Wall Thickness	High	Low	Med	High	Fig. 2-4
Relative Case Dry Weight ‡	Med	Low	Med	High	Fig. 2-6, 2-8 & Appendix

† Standardized relative to the length of the capsule chamber

‡ Standardized relative to the capsule wet weight

Table 2-9. Summary of the relative importance of genotypic versus environmental effects in explaining variation in capsule morphology among populations of *Nucella emarginata* in Barkley Sound. The actual data on which this table is based can be found in the figures and tables indicated in the right-hand column.

	INTERPOPULATION DIFFERENCES		
Trait	Mostly Genetic	Mostly Environmental	Figure/Table
Female Size at Maturity	X		Fig. 2-16
Seasonality of Capsule Production	X		Fig. 2-17
Capsule Chamber Length	†	X	Fig. 2-13, 2-14; Table 2-5, 2-6
Relative Chamber Width		X	Fig 2-13
Relative Plug Length	†	X	Fig. 2-14
Capsule Wall Thickness	†	X	Fig. 2-4, 2-10, 2-11, 2-12
# Embryos / Unit Volume	†	X	Table 2-5, 2-6

† Direct evidence of genetic effects also exists

Figure 2-1. Variation in the morphology of freshly deposited *Nucella emarginata* egg capsules collected from ten sites separated along a wave-exposure gradient in Barkley Sound in June 1991. Each histogram represents the mean (± SE) of ten clutches per site (except Cape Beale, where n = 9), and each clutch represents the average measurements of three capsules. ANOVA was used to compare differences in mean capsular dimensions, volumes or shape indices among sites (see Table 2-1). Chamber widths are expressed as adjusted means (following ANCOVA), because chamber width was related to chamber length for most sites (see Fig. 2-3; Table 2-2). The results of Tukey *a posteriori* multi-comparison tests are indicated above each histogram: sites not connected with a horizontal line are significantly different from one another at $\alpha = 0.05$. Sites are abbreviated as: GR = Grappler Inlet, RI = Ross Islet, KB = Kelp Bay, DX = Dixon Island, SP = Self Point, WZ = Wizard Rock, VP = Voss Point, KP = Kirby Point, CB = Cape Beale, FG = Folger Island. Sites are ranked along the x-axis in order of increasing wave-exposure.

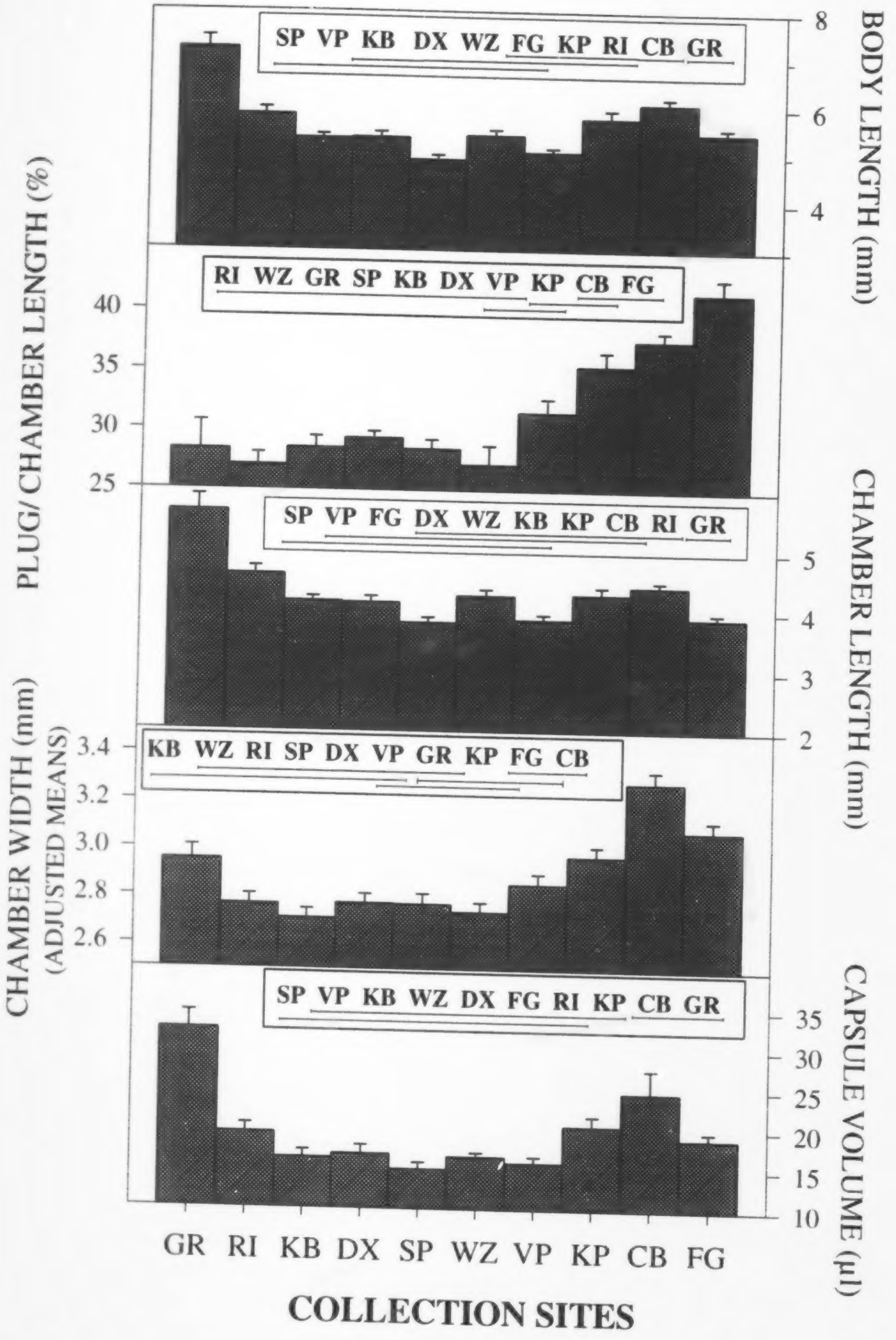

SP VP KB DX WZ FG KP RI CB GR
BODY LENGTH (mm)
8
6
4
RI WZ GR SP KB DX VP KP CB FG
PLUG/ CHAMBER LENGTH (%)
40
35
30
25
SP VP FG DX WZ KB KP CB RI GR
CHAMBER LENGTH (mm)
5
4
3
2
KB WZ RI SP DX VP GR KP FG CB
CHAMBER WIDTH (mm)
(ADJUSTED MEANS)
3.4
3.2
3.0
2.8
2.6
SP VP KB WZ DX FG RI KP CB GR
CAPSULE VOLUME (µl)
35
30
25
20
15
10
GR RI KB DX SP WZ VP KP CB FG
COLLECTION SITES

Figure 2-2. Variation in the morphology of freshly deposited *Nucella emarginata* egg capsules collected from four sites in April - June 1992. Each histogram represents the mean (± SE) of 50 capsules (1 capsule per clutch) collected from each population (except Cape Beale where n = 51 capsules). ANOVA was used to compare differences in mean capsular dimensions, volumes or indices among sites (see Table 2-1). Chamber widths are expressed as adjusted means (following ANCOVA), because chamber width was related to chamber length for most sites (see Fig. 2-3). The results of Tukey *a posteriori* multi-comparison tests are indicated above each histogram: sites not connected with a horizontal line are significantly different from one another at $\alpha = 0.05$.

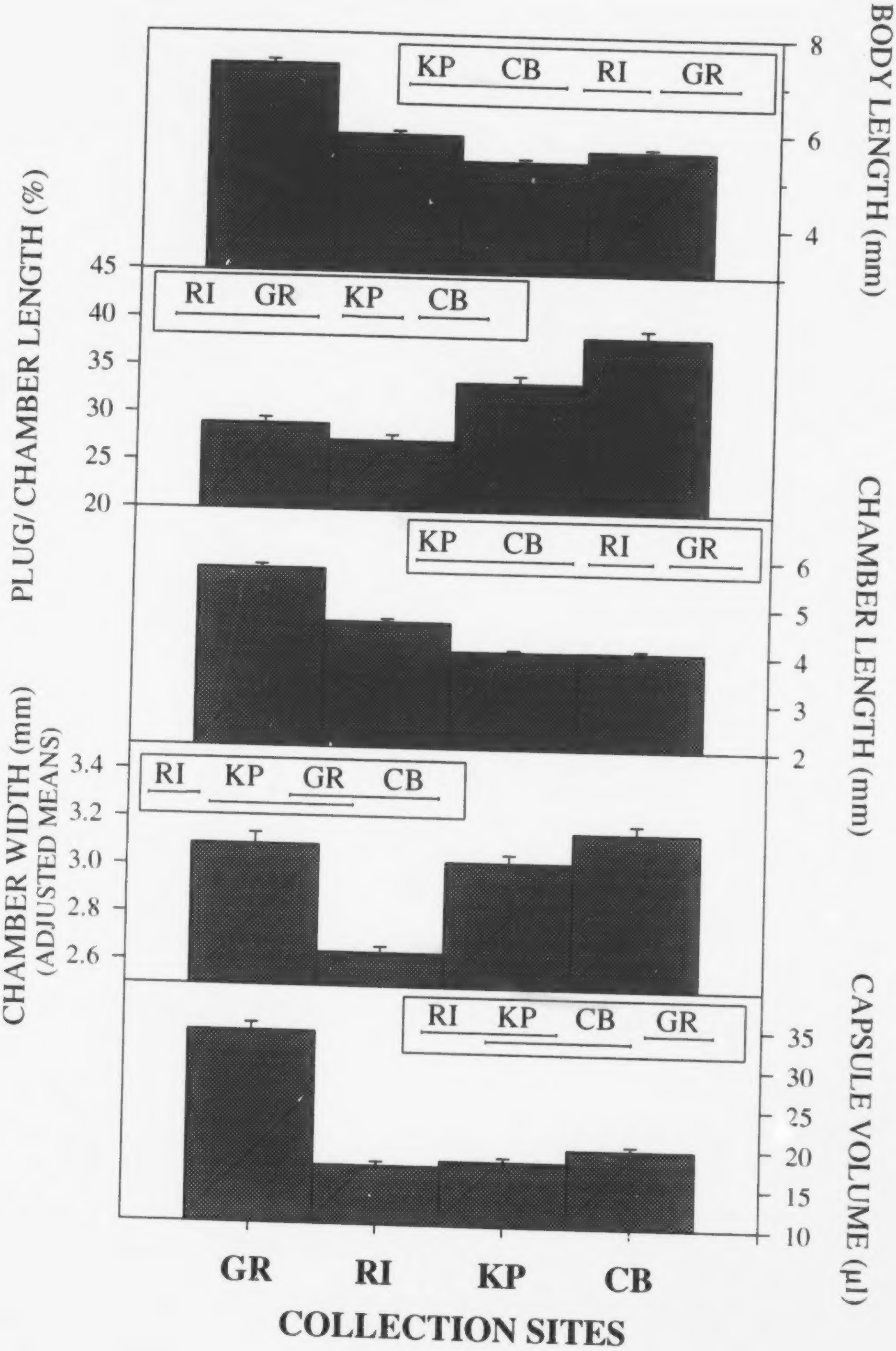

BODY LENGTH (mm)
8
6
4
KP CB RI GR
PLUG/ CHAMBER LENGTH (%)
45
40
35
30
25
20
RI GR KP CB
CHAMBER LENGTH (mm)
6
5
4
3
2
KP CB RI GR
CHAMBER WIDTH (mm)
(ADJUSTED MEANS)
3.4
3.2
3.0
2.8
2.6
RI KP GR CB
CAPSULE VOLUME (µl)
35
30
25
20
15
10
RI KP CB GR
GR
RI
KP
CB
COLLECTION SITES

Figure 2-3. Relations between chamber width and chamber length for *Nucella emarginata* capsules collected from all 10 sites in the summer of 1991. Each symbol represents the mean of 3 - 6 capsules per clutch. Hollow symbols refer to capsules collected from the three most wave-exposed sites (Kirby Point, Cape Beale, Folger Island), filled symbols represent capsules collected from all other sites. Least-squares linear regression equations and sample sizes for each site are given in Table 2-2.

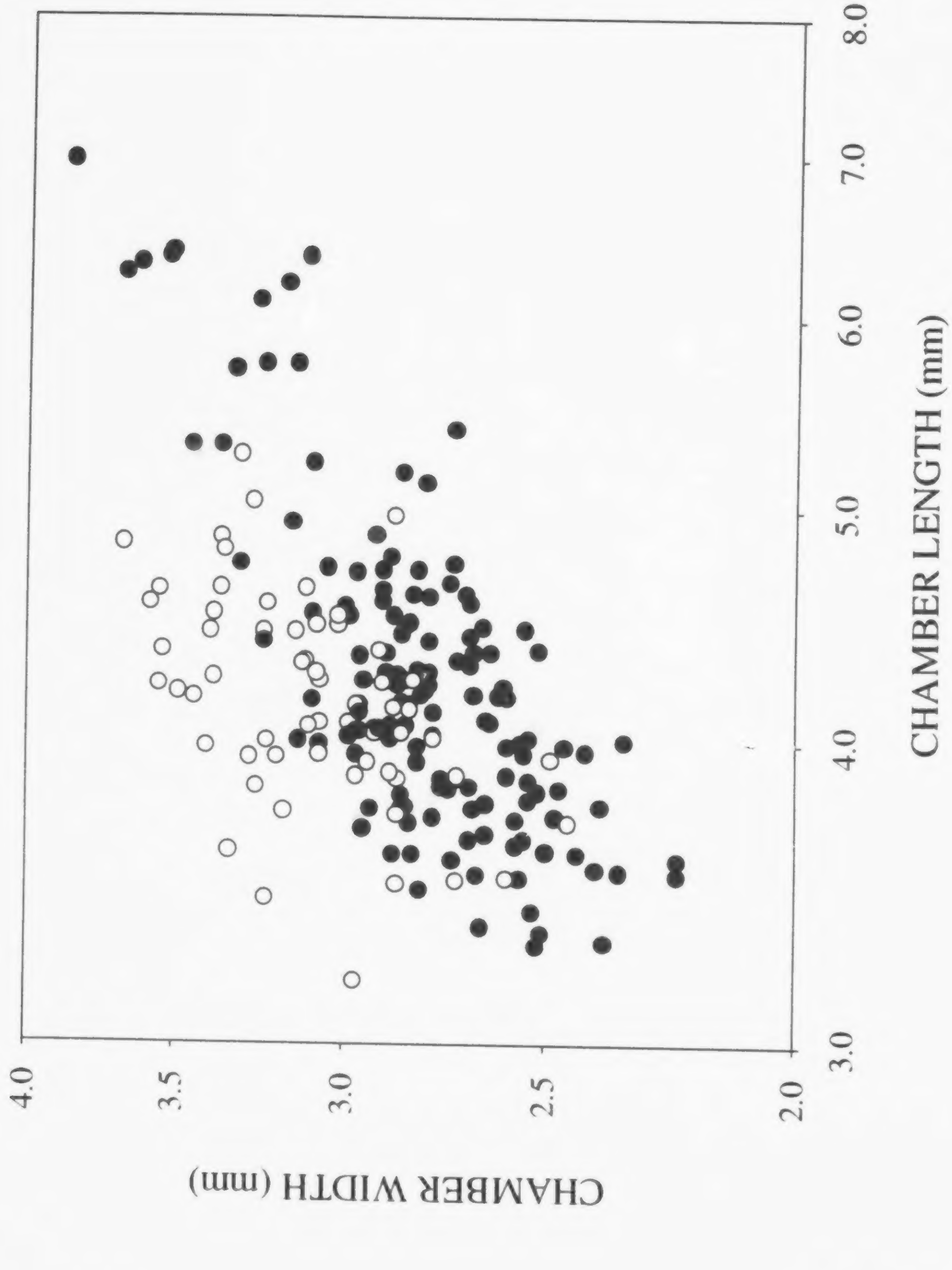

CHAMBER WIDTH (mm)
4.0
3.5
3.0
2.5
2.0
CHAMBER LENGTH (mm)
3.0
4.0
5.0
6.0
7.0
8.0

Figure 2-4. Mean (± SE) wall thickness of *Nucella emarginata* capsules collected from 10 different clutches at each of 10 sites separated along a gradient of wave-exposure in Barkley Sound in the summer of 1990 (n = 1 capsule/clutch x 10 clutches per site), and 1991 (n = 3 capsules/clutch x 10 clutches per site). Also included for comparison is the mean wall thickness of capsules collected at Grappler Inlet, Ross Islet and Seppings Island (adjacent to Kirby Pt.) sites in 1988 (n = 1 capsule/clutch x 8 clutches). Mean capsule wall thickness was compared among sites and between years using a two-way ANOVA (mixed model). The results illustrated a significant site-effect ($F = 8.01$, $P < 0.025$, df = 9, 9), a non-significant year-effect ($F = 0.73$, $P = 0.39$, df = 1, 174), and a significant interaction between sites and years ($F = 301.46$, $P < 0.001$, df = 9, 174).

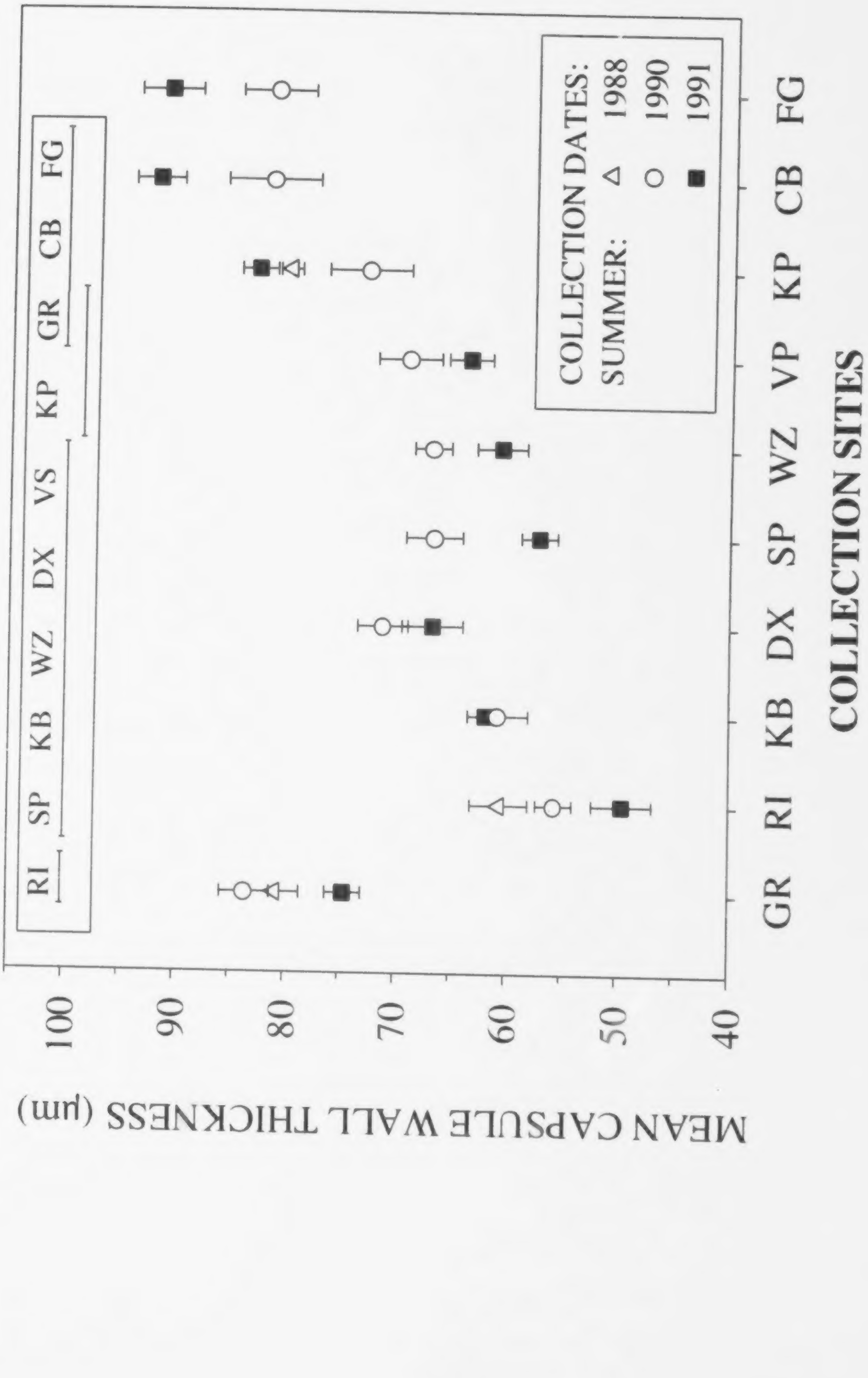

MEAN CAPSULE WALL THICKNESS (μm)
100
90
80
70
60
50
40
COLLECTION SITES
GR
RI
KB
DX
SP
WZ
VP
KP
CB
FG
RI SP KB WZ DX VS KP GR CB FG
COLLECTION DATES:
SUMMER:
△ 1988
○ 1990
■ 1991

Figure 2-5. Mean (± SE) wall thickness of capsules collected from the highest and lowest transects along which capsules were present at Ross Islet and Kirby Point in June 1991. Sample sizes range from 9 - 10 capsules collected from separate clutches along each transect.

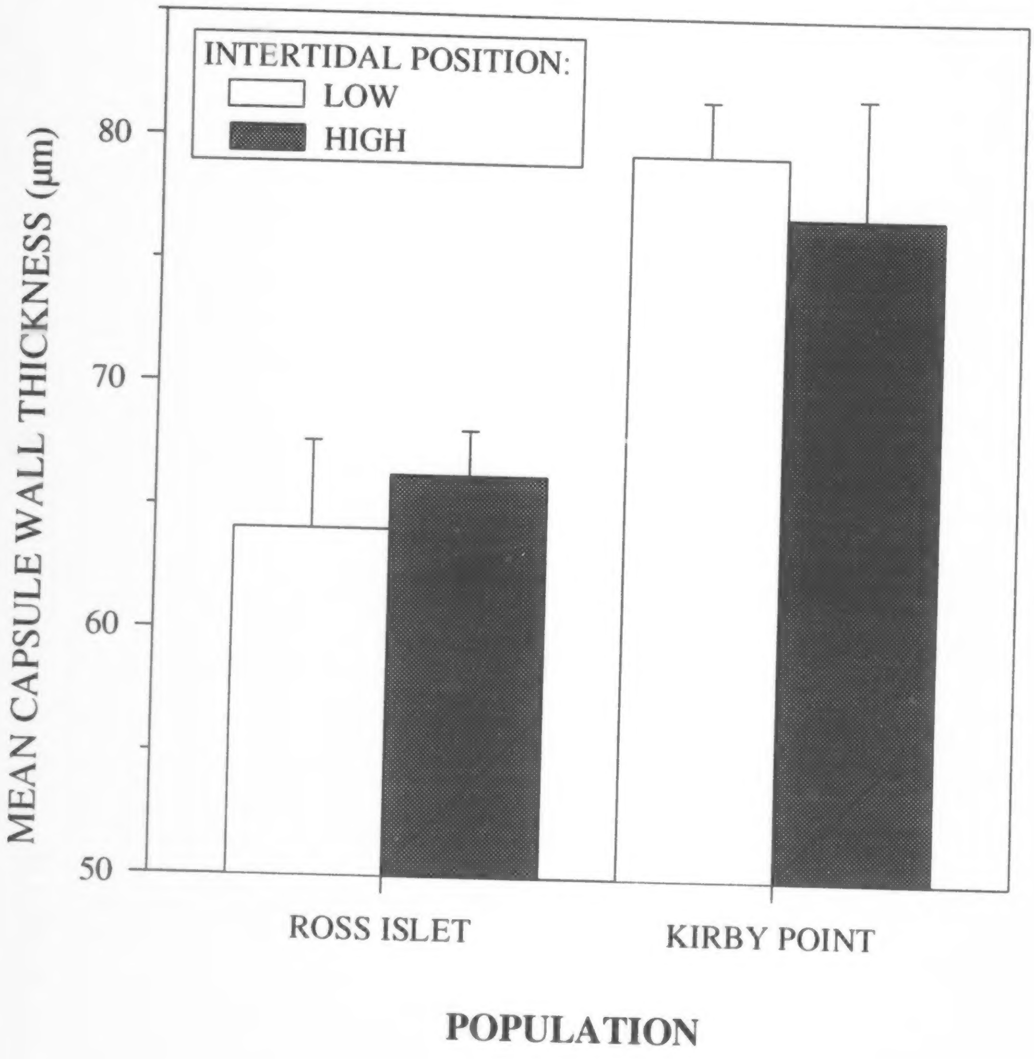


Figure 2-6. The relationship between a) log (case dry weight) versus log (whole wet weight) and b) log (whole dry weight) versus log (whole wet weight), for capsules collected from all ten sites in the summer of 1991. Ten capsules were sampled per site (n = 1 capsule/clutch for each of ten clutches) and, when possible, additional capsules were also sampled from other clutches (see Table 2-3 for actual sample sizes). Lines indicate least-square linear regressions for individual sites.

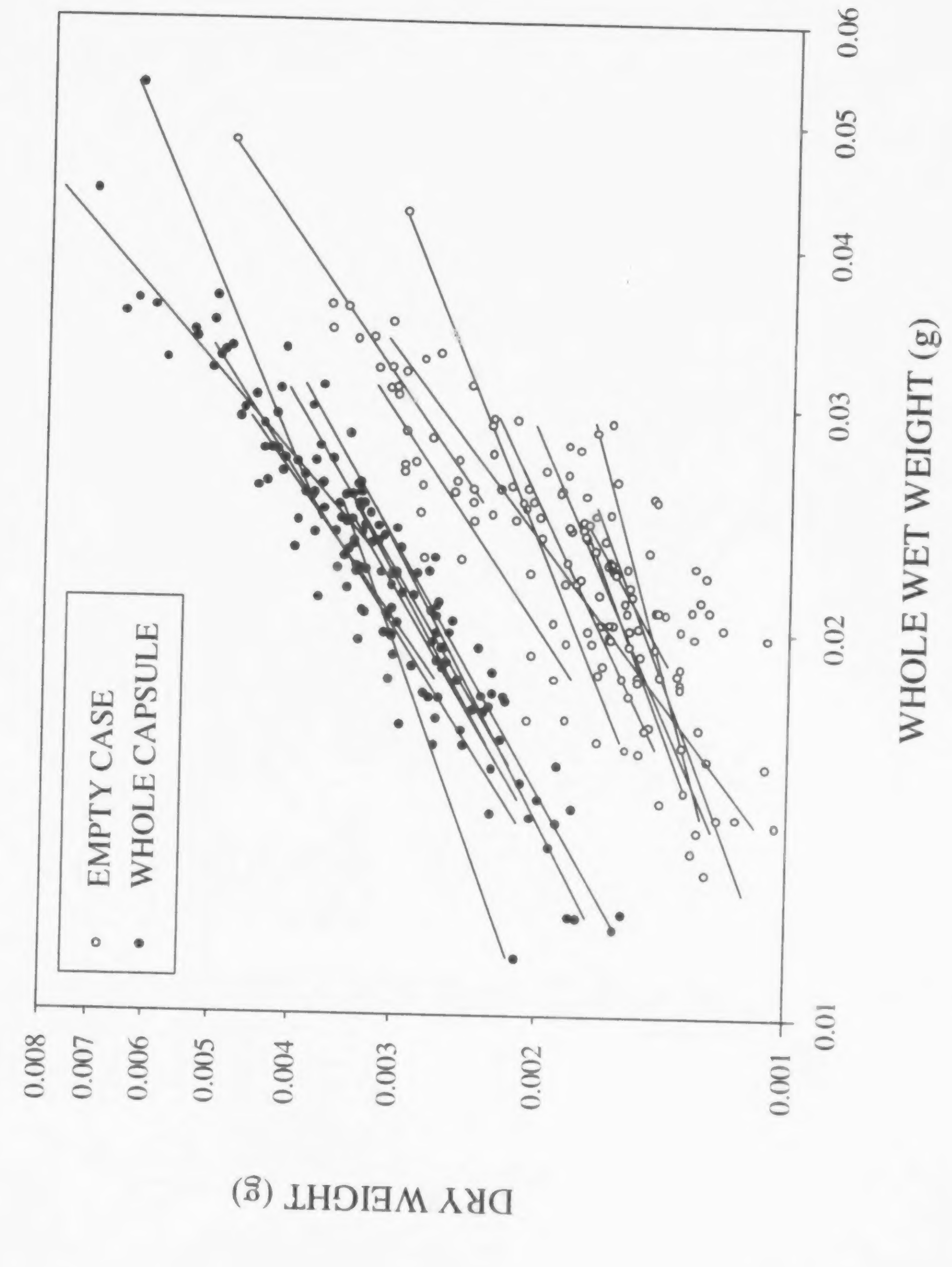

EMPTY CASE
WHOLE CAPSULE
DRY WEIGHT (g)
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
WHOLE WET WEIGHT (g)
0.01
0.02
0.03
0.04
0.05
0.06

Figure 2-7. Associations between mean case dry weight and mean capsule wall thickness, for capsules (wet weight = 0.025 g) collected from ten sites in Barkley Sound in 1991. Mean dry weights were determined using site-specific regression equations shown in Fig. 2-6 and Table 2-3. A common wet weight of 0.025 g was chosen for comparison because capsules from all sites spanned this capsule weight. Mean capsule wall thickness was determined from a sample of ten clutches of capsules collected from each population in the summer of 1991 (see Fig. 2-4). The regression equation for the relationship is: $Y = 0.0196 (\pm 0.00180) X + 0.6178 (\pm 0.12870)$, $r^2 = 0.937$, $n = 10$.

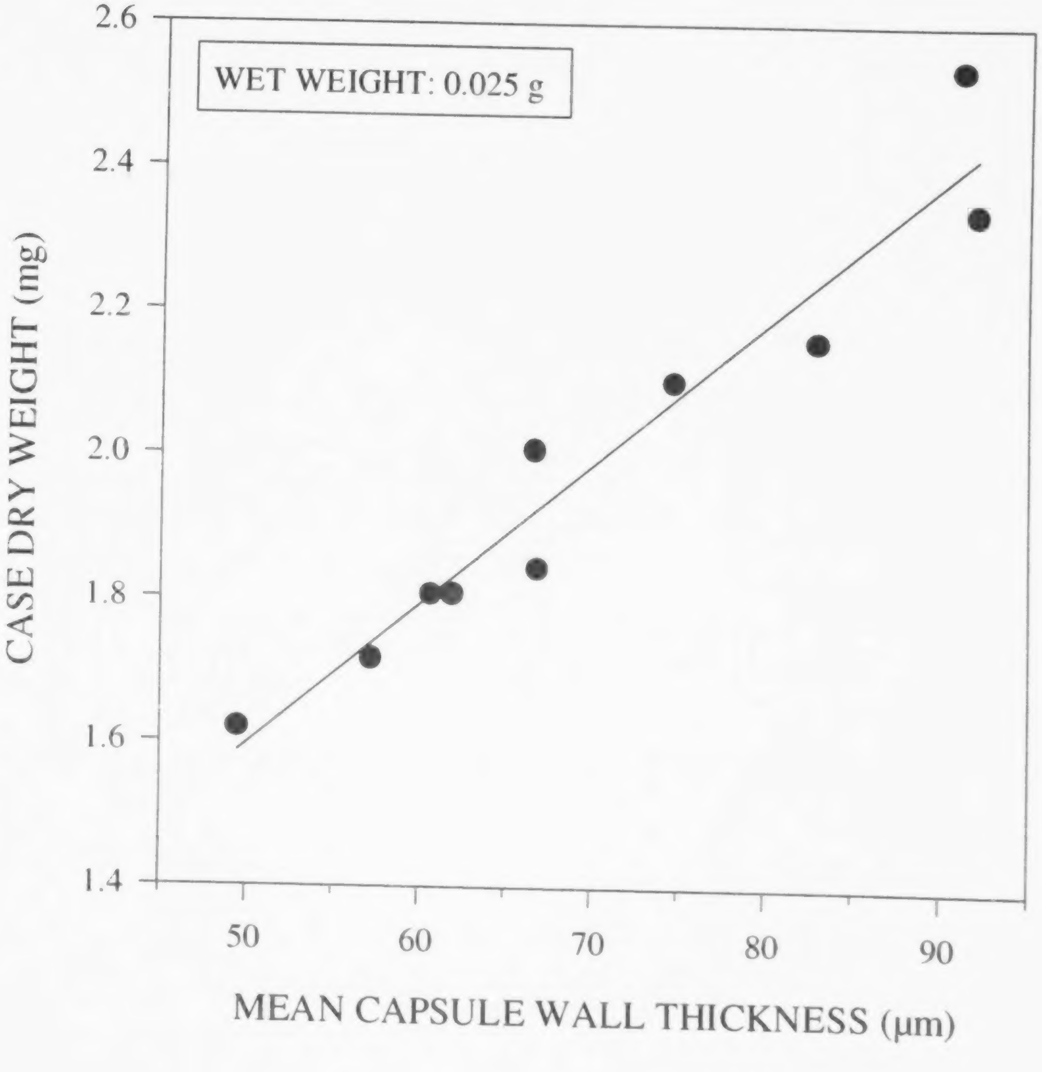


Figure 2-8. Logarithmic relationships between a) case dry weight and whole wet weight and b) whole dry weight and whole wet weight, for capsules collected from four different sites in Barkley Sound in 1992 (see Table 2-3 for least-squares linear regression equations and sample sizes). ANCOVA was used to compare these relationships among study sites for dry weights of capsular cases and whole capsules: Capsular cases: F(slopes) = 0.76, df = 3, 92, P = 0.52; F(elevations) = 29.09, df = 3, 92, P < 0.001; Tukey *a posteriori* test on adjusted means: (RI = GR = KP) < CB; Whole capsule dry weights: F(slopes) = 1.41, df = 3, 95, P = 0.24; F(elevations) = 19.40, df = 3, 95, P < 0.001; Tukey *a posteriori* comparisons: GR < (RI = KP) < (KP = CB).

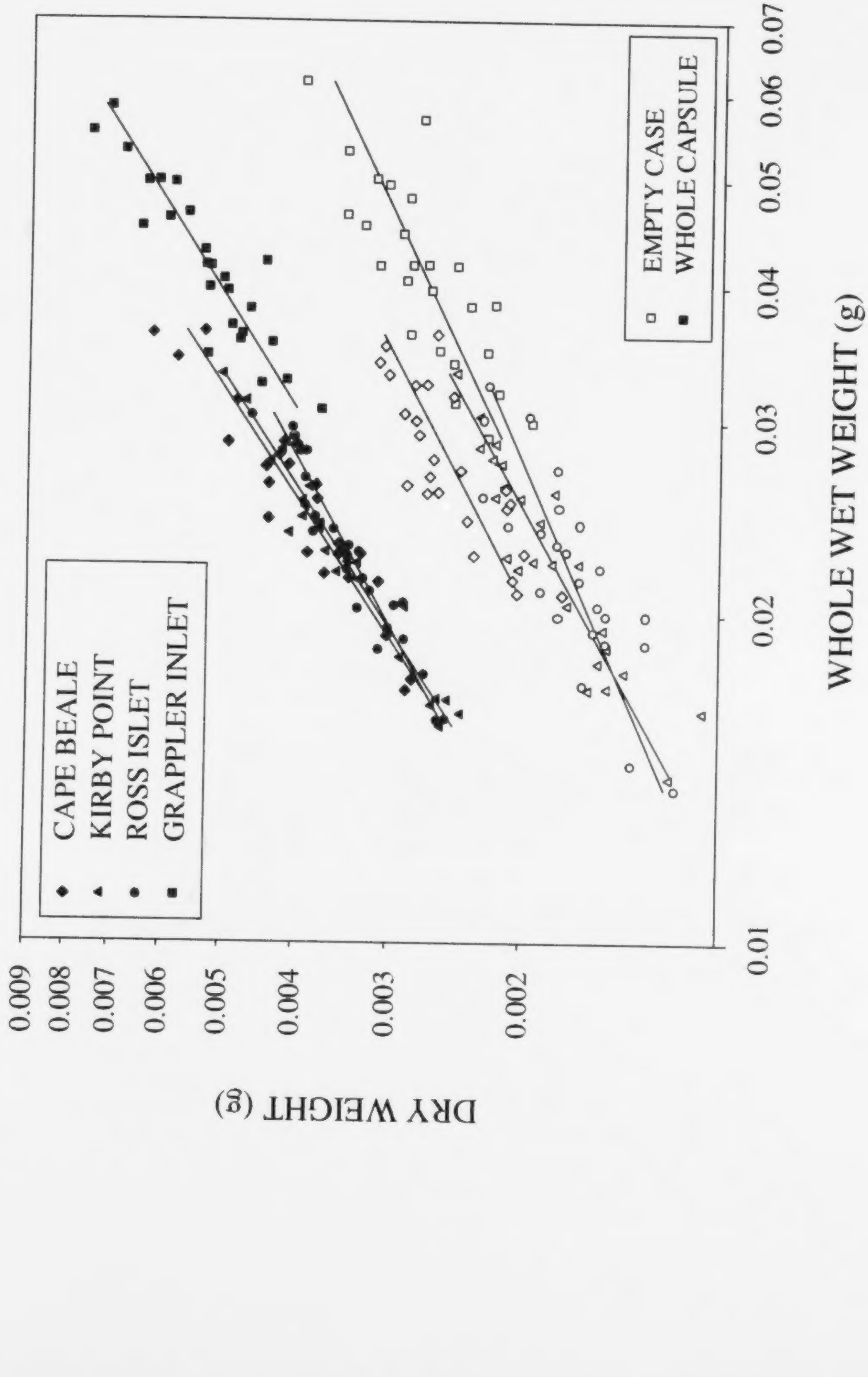

DRY WEIGHT (g)
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
WHOLE WET WEIGHT (g)
0.01
0.02
0.03
0.04
0.05
0.06
0.07
CAPE BEALE
KIRBY POINT
ROSS ISLET
GRAPPLER INLET
EMPTY CASE
WHOLE CAPSULE

Figure 2-9. The proportional contribution of the case to the dry weight of whole *N. emarginata* capsules collected from ten sites in 1991 and four sites in 1992. Comparisons were made for three capsule wet weights of 0.02, 0.025 and 0.03 g. Dry weights of cases and whole capsules were determined from these wet weights using the site-specific regression equations given in Table 2-3.

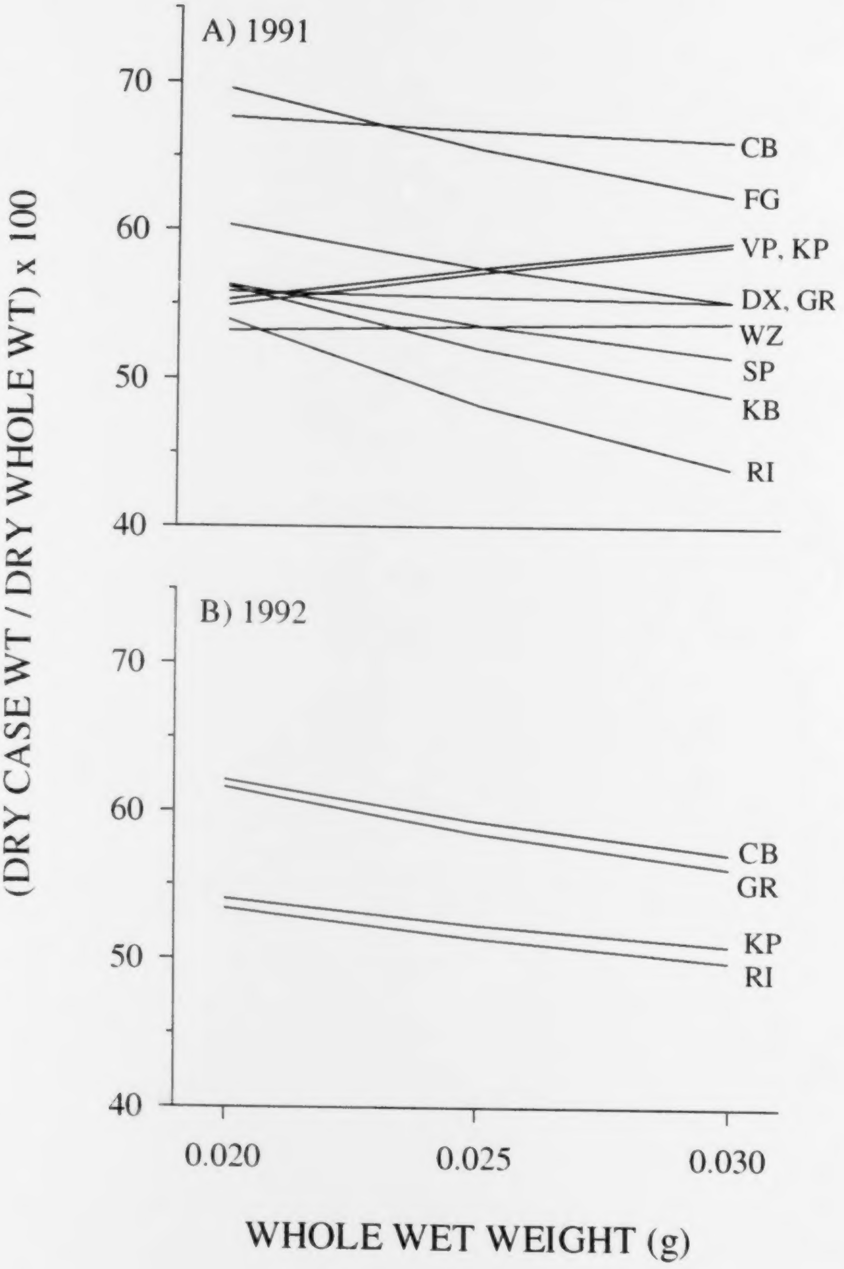


Figure 2-10. Mean wall thickness (± SE) of sequential clutches of capsules produced by five individual female *Nucella emarginata* collected from Grappler Inlet and Ross Islet when confined to laboratory conditions. Day 0 refers to the date that snails were collected from their respective field populations. Five capsules were sectioned from each female for each date that capsules were collected. Because cages were only checked at two-three week intervals, the actual period that capsules were spawned in the laboratory may have been two-three weeks earlier than indicated.

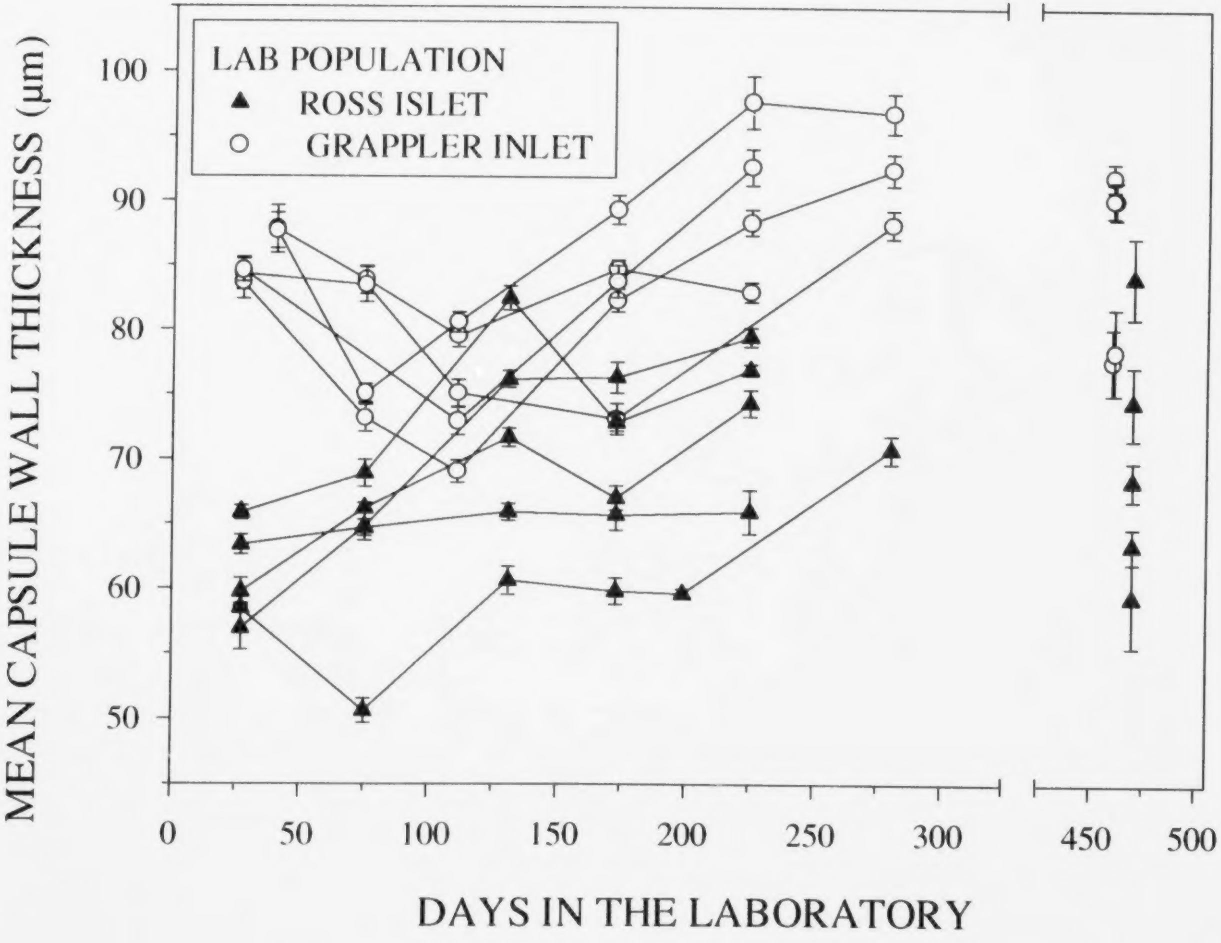

LAB POPULATION
ROSS ISLET
GRAPPLER INLET
MEAN CAPSULE WALL THICKNESS (µm)
100
90
80
70
60
50
0
50
100
150
200
250
300
450
500
DAYS IN THE LABORATORY

Figure 2-11. Mean wall thickness (± SE) of capsules produced by F_1 snails raised in the laboratory compared to capsules laid by field-collected P_1 snails under a) field conditions and b) laboratory conditions, for three different source populations of *Nucella emarginata* (Grappler Inlet, Ross Islet, Folger Island). Field-collected capsules represent a sample of ten capsules (1 capsule/clutch) collected from each study site in June 1990. Capsules spawned in the laboratory by P_1 snails were produced by five snails (five capsules/clutch/individual) within a two month period following their collection from the field in June 1990 (Folger snails are not included). Capsules spawned by F_1 snails represent a clutch of capsules produced by snails born and raised entirely within the laboratory. These capsules are separated according to the parentage of F_1 snails (family #1 vs. #2: i.e. siblings *vs.* non-siblings). Each symbol represents the mean of 3-5 capsules (± SE) sectioned per female F_1 snail. The wall thickness of capsules spawned among the three laboratory populations of F_1 snails was compared using a nested ANOVA, with variation among families and sibling females nested within each population. The results of this analysis were: F(population) = 0.83, df = 2, 90, $P > 0.25$; F(family) = 1.23, df = 3, 90, $P > 0.25$; F(siblings) = 10.77, df = 20, 90, $P < 0.001$.

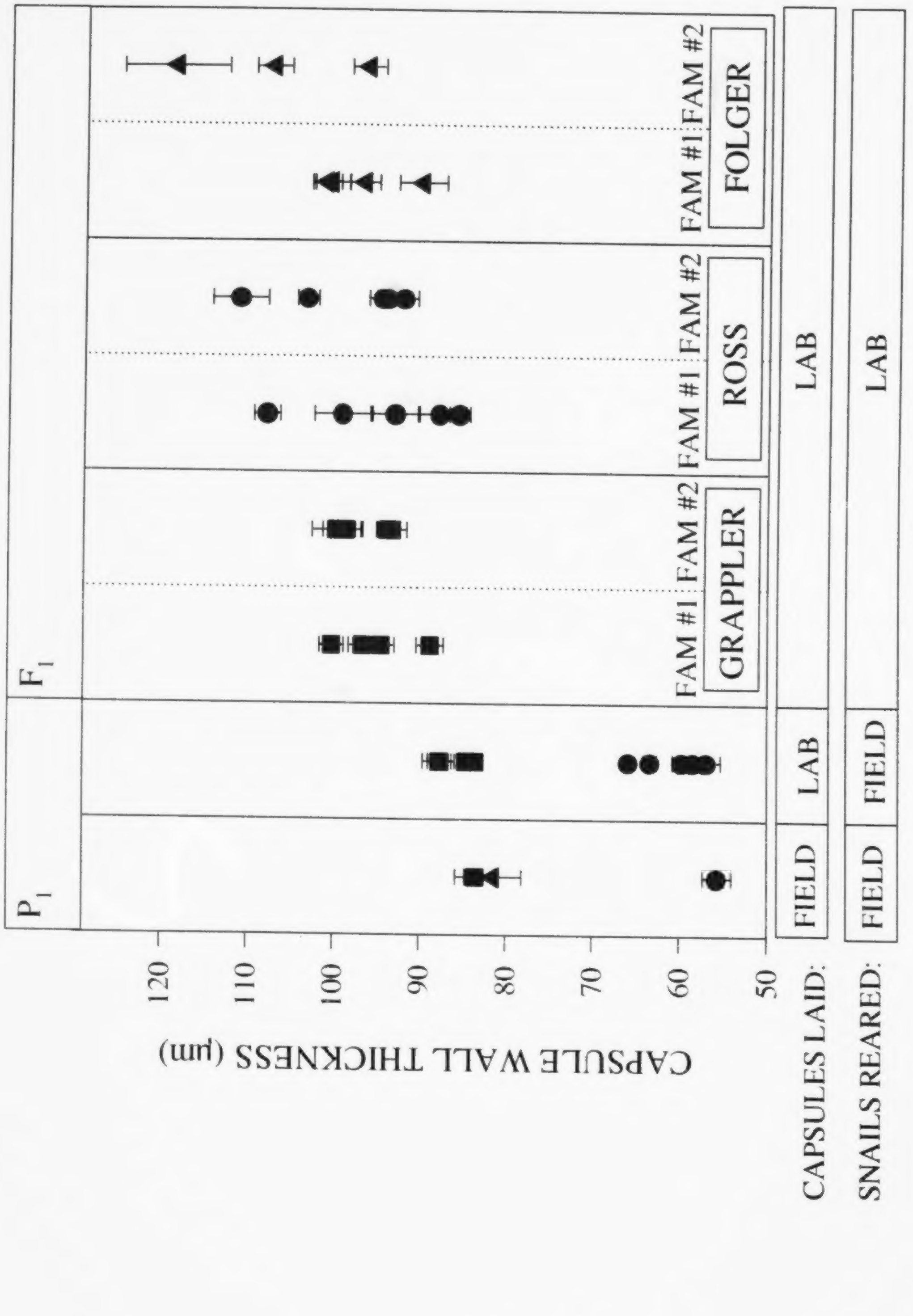

CAPSULE WALL THICKNESS (µm)
120
110
100
90
80
70
60
50
P1
F1
FAM #1
FAM #2
GRAPPLER
ROSS
FOLGER
CAPSULES LAID:
FIELD
LAB
LAB
SNAILS REARED:
FIELD
FIELD
LAB

Figure 2-12. Comparison of the wall thickness of capsules produced by sibling female snails when raised in slightly different culture conditions. "Isolated" females (i.e., solid symbols) were separated from group rearing cages in April, 1991, paired with male snails, and then allocated to their own mesh-paneled cages. "Grouped" snails were kept in group rearing cages until November 1991, after which they were raised in their own mesh-paneled cages, as above. Four - five sibling females were examined per culture condition for each of two families for both Grappler Inlet and Ross Islet F_1 populations. Each symbol represents the mean (± SE) of 3-5 capsules sectioned per female F_1 snail. Different rearing conditions had a significant effect on the wall thickness of Ross Islet capsules (Fig 2-11; 2-way ANOVA: F(rearing) = 37.11, df = 1,16, $P < 0.001$; F(family) = 2.03, df = 1, 16, $P > 0.10$; F(interaction) = 0.00, df = 1, 15, P = 0.99). Although rearing conditions did not significantly affect the capsules produced by Grappler Inlet snails (2-way ANOVA: F(rearing) = 0.11, df = 1,14, $P > 0.25$; F(family) = 0.59, df = 1, 14, $P > 0.25$; F(interaction) = 10.62, df = 1, 14, P = 0.006), offspring from Family #2 exhibited a treatment response similar to both Ross Islet families.

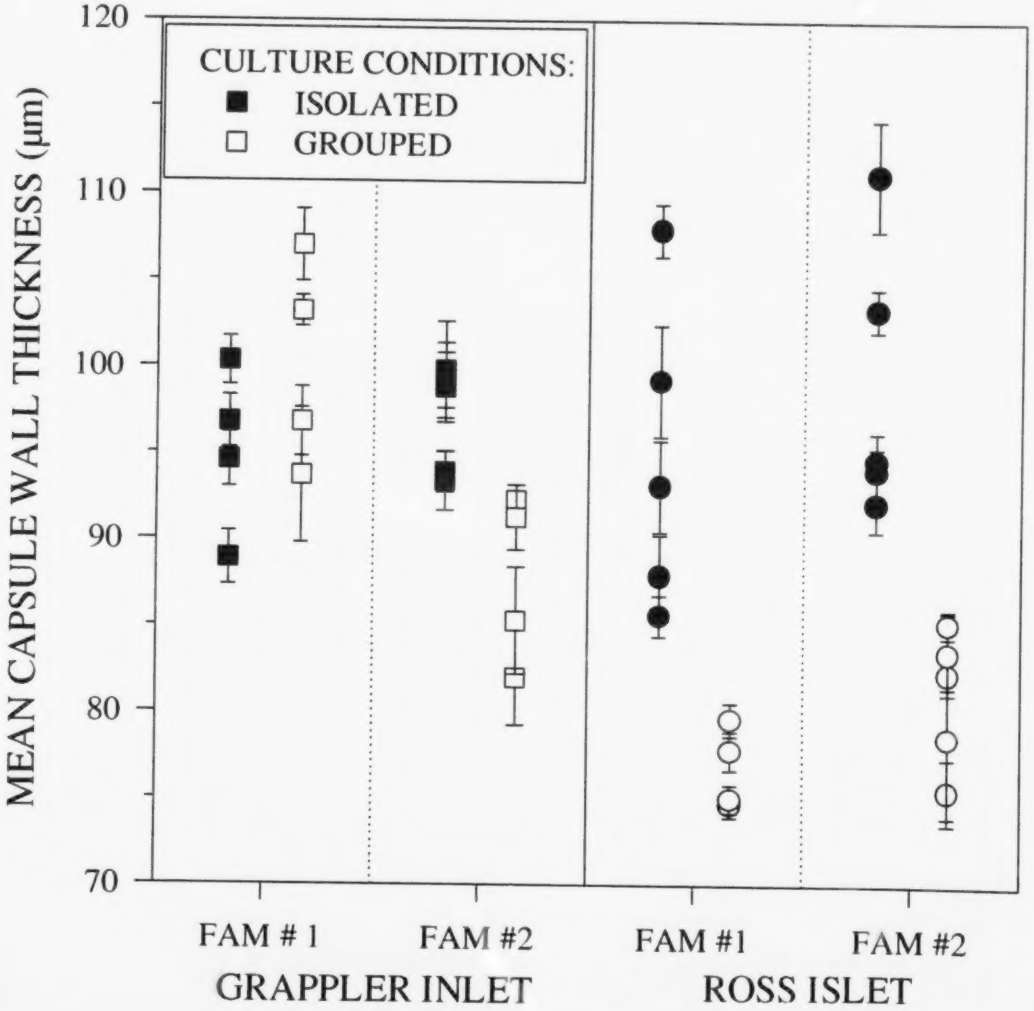


Figure 2-13. Associations between chamber width and chamber length for field-collected capsules and capsules spawned by laboratory-reared F_1 snails from Grappler Inlet, Ross Islet, and Folger Island populations. Each symbol represents the mean of at least three capsules per clutch. Field collected capsules were pooled over years where possible. Sample sizes and regression equations are given in Table 2-4.

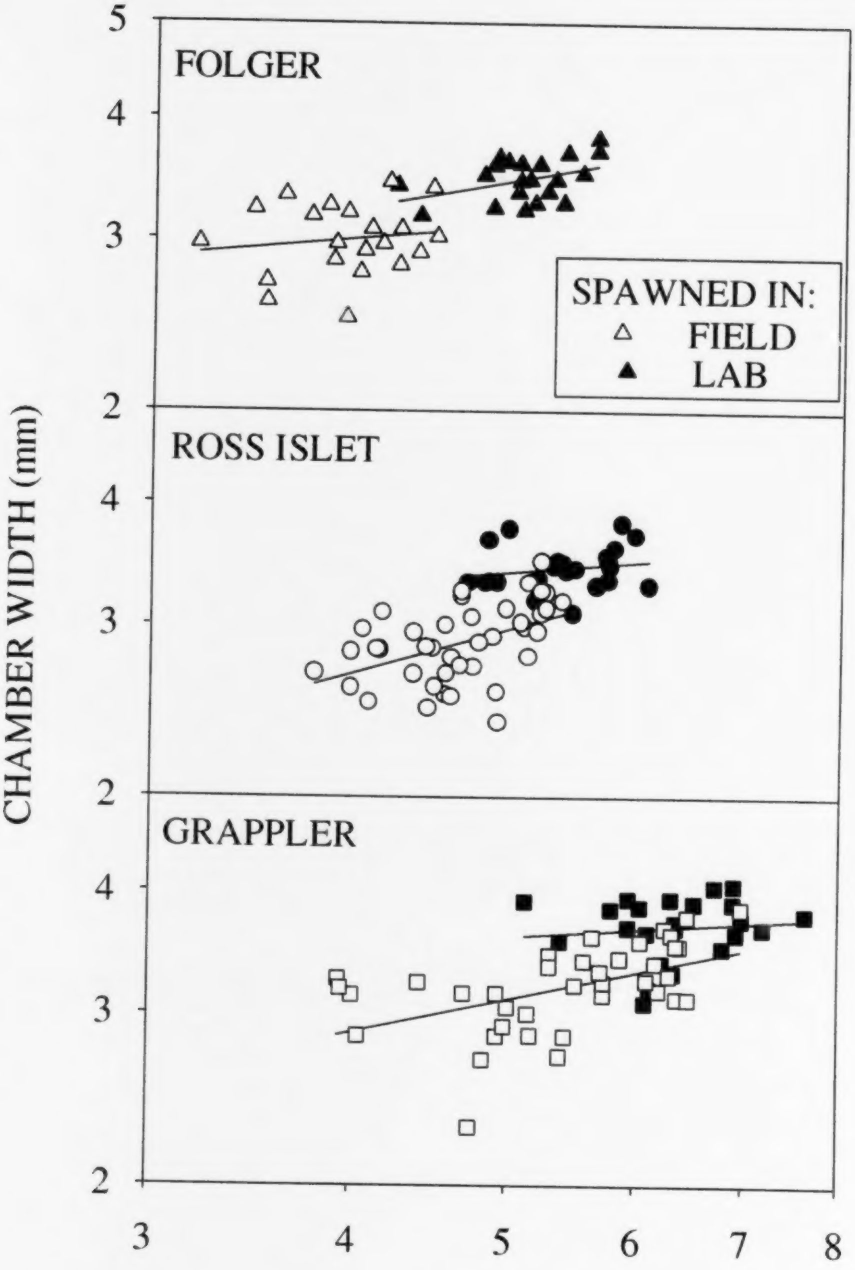

FOLGER
ROSS ISLET
GRAPPLER
SPAWNED IN:
△ FIELD
▲ LAB
CHAMBER WIDTH (mm)
CHAMBER LENGTH (mm)
5
4
3
2
4
3
2
4
3
2
3
4
5
6
7
8

Figure 2-14. Associations between plug size and chamber length for field-collected capsules and capsules spawned by laboratory-reared F_1 snails from Grappler Inlet, Ross Islet, and Folger Island populations. Each symbol represents the mean of at least three capsules per clutch. Field collected capsules were pooled over years where possible. Sample sizes and regression equations are given in Table 2-4.

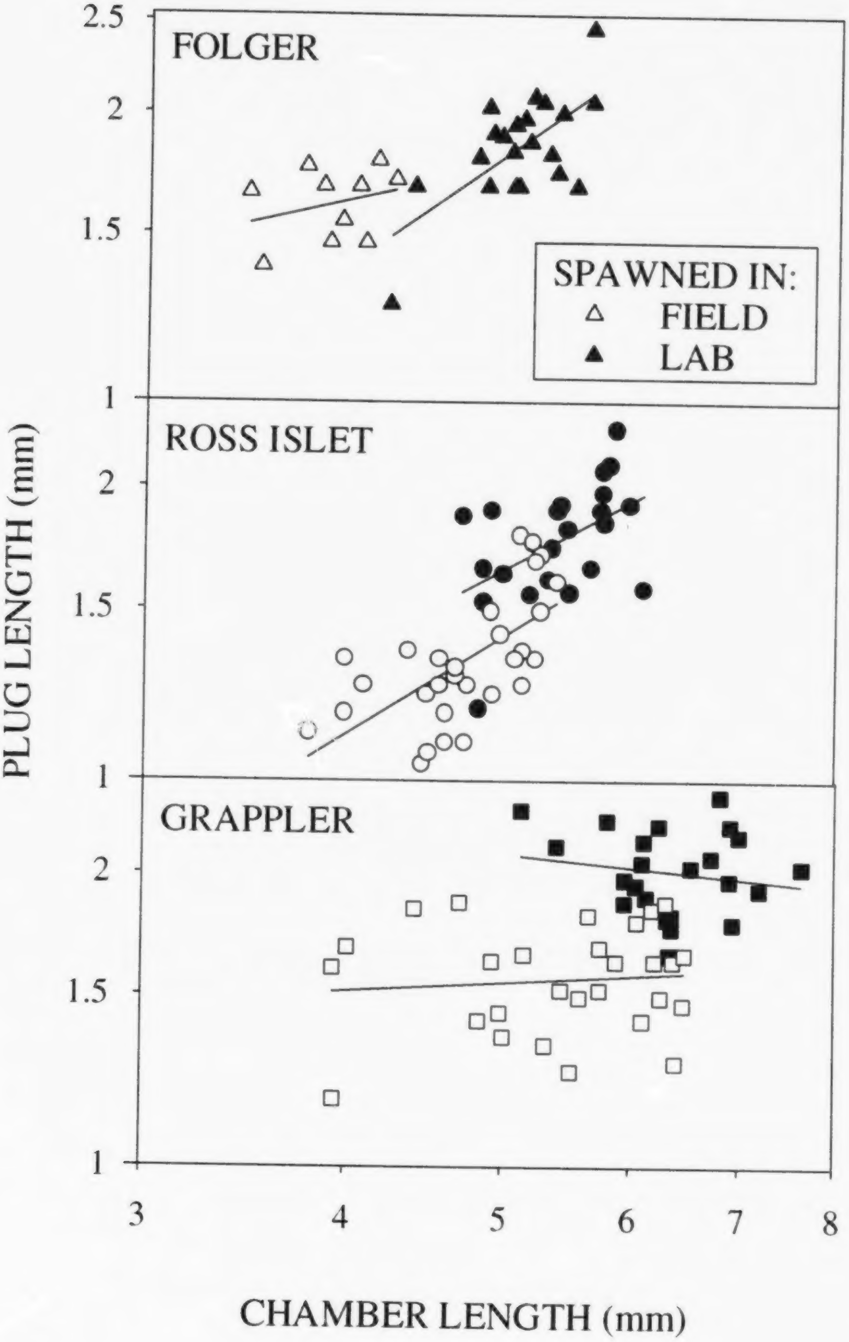

FOLGER
ROSS ISLET
GRAPPLER
SPAWNED IN:
△ FIELD
▲ LAB
PLUG LENGTH (mm)
CHAMBER LENGTH (mm)
2.5
2
1.5
1
2
1.5
1
2
1.5
1
3
4
5
6
7
8

Figure 2-15. Estimated ash-free dry weights of barnacle flesh (*Balanus glandula*) consumed per cage by *Nucella emarginata* from three different laboratory populations exposed to two different ration treatments: 100% ration and 33% ration. Numbers of barnacles consumed were determined over a 10 d period for snails in both treatment groups (n = 9 cages per treatment group). Because snails in 100% ration treatment group were exposed to food for 3 times as long as snails in the 33% ration group, the amount of food eaten over 10 days in the 100% ration group was multiplied by three to estimate the total food consumption over the full 30 d cycle.

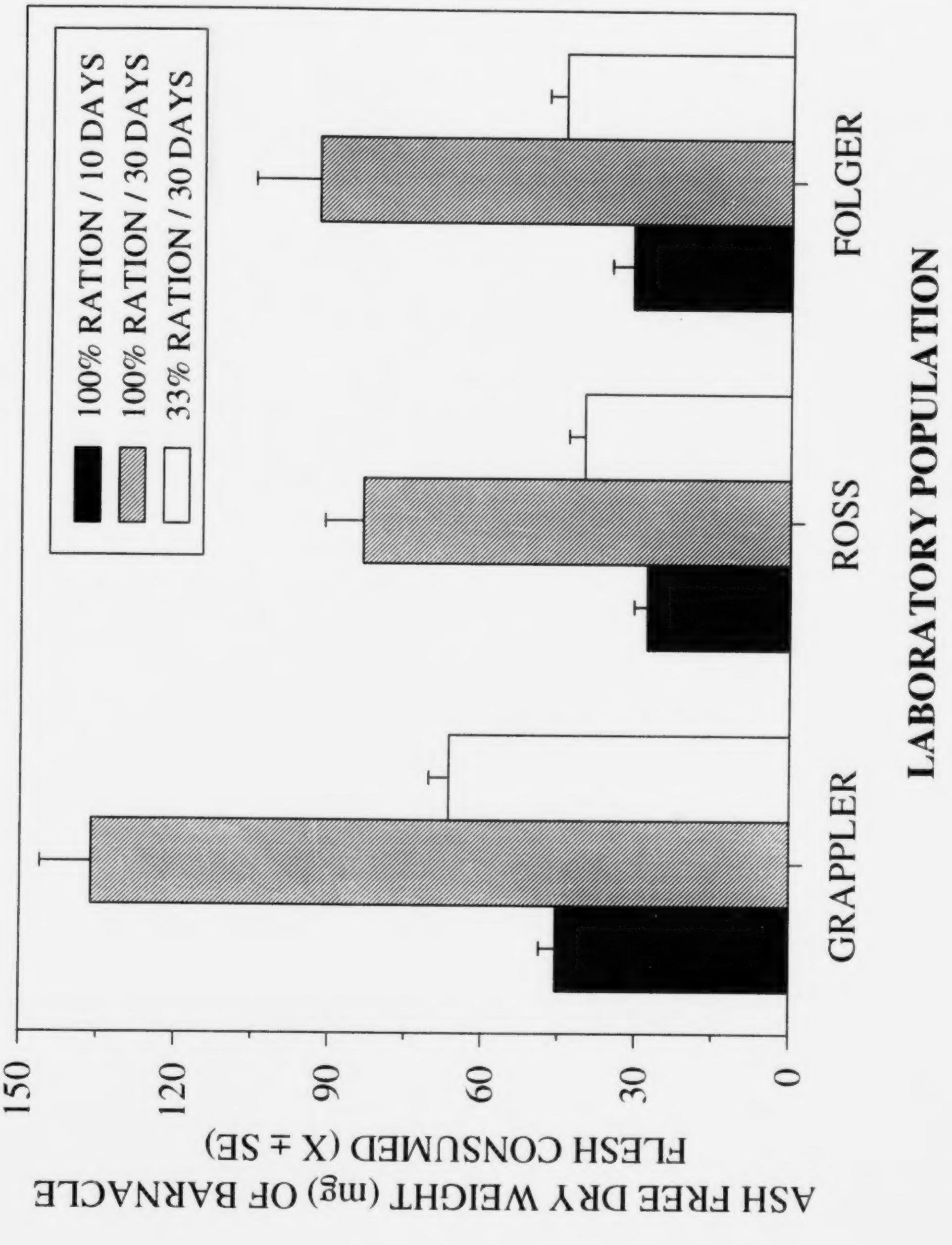

ASH FREE DRY WEIGHT (mg) OF BARNACLE FLESH CONSUMED (X ± SE)
150
120
90
60
30
0
GRAPPLER
ROSS
FOLGER
LABORATORY POPULATION
100% RATION / 10 DAYS
100% RATION / 30 DAYS
33% RATION / 30 DAYS

Figure 2-16. Growth of female *Nucella emarginata* snails from three laboratory populations (Grappler Inlet, Ross Islet, or Folger Island) when raised on different diet rations. Data are expressed as mean shell length (± SE) for nine female snails sampled from each ration treatment condition for each population. Day "0" refers to the date on which snails were removed from communal holding cages and isolated in their own individual mesh-paneled containers. The vertical dotted line on day 111 indicates the date on which snails were subjected to different food ration treatment conditions: either 100% (solid symbols) or 33% (open symbols) food ration (66% ration data have been removed for clarity). Capsules were collected from spawning female snails after > 6-months exposure to these treatment conditions (day 300). A three-way ANOVA comparing shell length between dates (Day 111 vs. Day 332), laboratory populations (Grappler, Ross or Folger), and food ration (33%, 66%, 100%), indicated a significant effect of date ($F = 4.17$, $P = 0.043$, $df = 1, 144$), and population ($F = 297.50$, $P < 0.001$, $df = 2, 144$), but not of ration treatment ($F = 0.13$, $P = 0.0875$, $df = 2, 144$). There were no significant interaction effects resulting from this analysis.

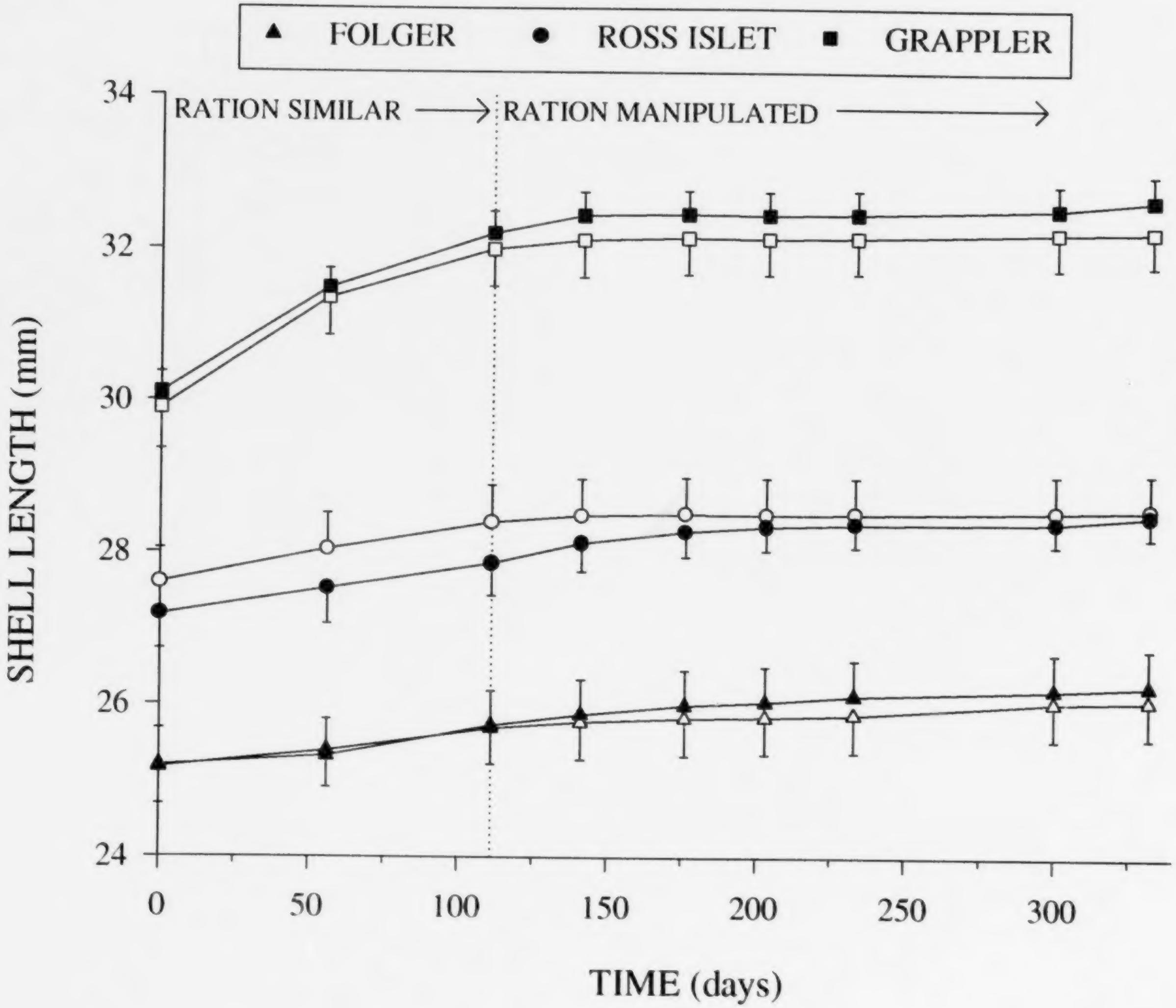

▲ FOLGER
● ROSS ISLET
■ GRAPPLER
RATION SIMILAR ⟶
RATION MANIPULATED ⟶
SHELL LENGTH (mm)
34
32
30
28
26
24
TIME (days)
0
50
100
150
200
250
300

Figure 2-17. Mean thickness of capsule walls (± SE) collected from three laboratory (F_1) populations (Grappler Inlet, Ross Islet, and Folger Island) prior to and after rearing snails for > 6 months on 100% ration and 33% ration treatments. Three capsules were sampled from each female and five females were selected from each treatment category. Capsules from the same females were selected for the post-treatment examination. The results of a three-way ANOVA comparing ration treatment, laboratory population, and spawning date (i.e. pre- versus post-treatment), are shown in Table 2-6i.

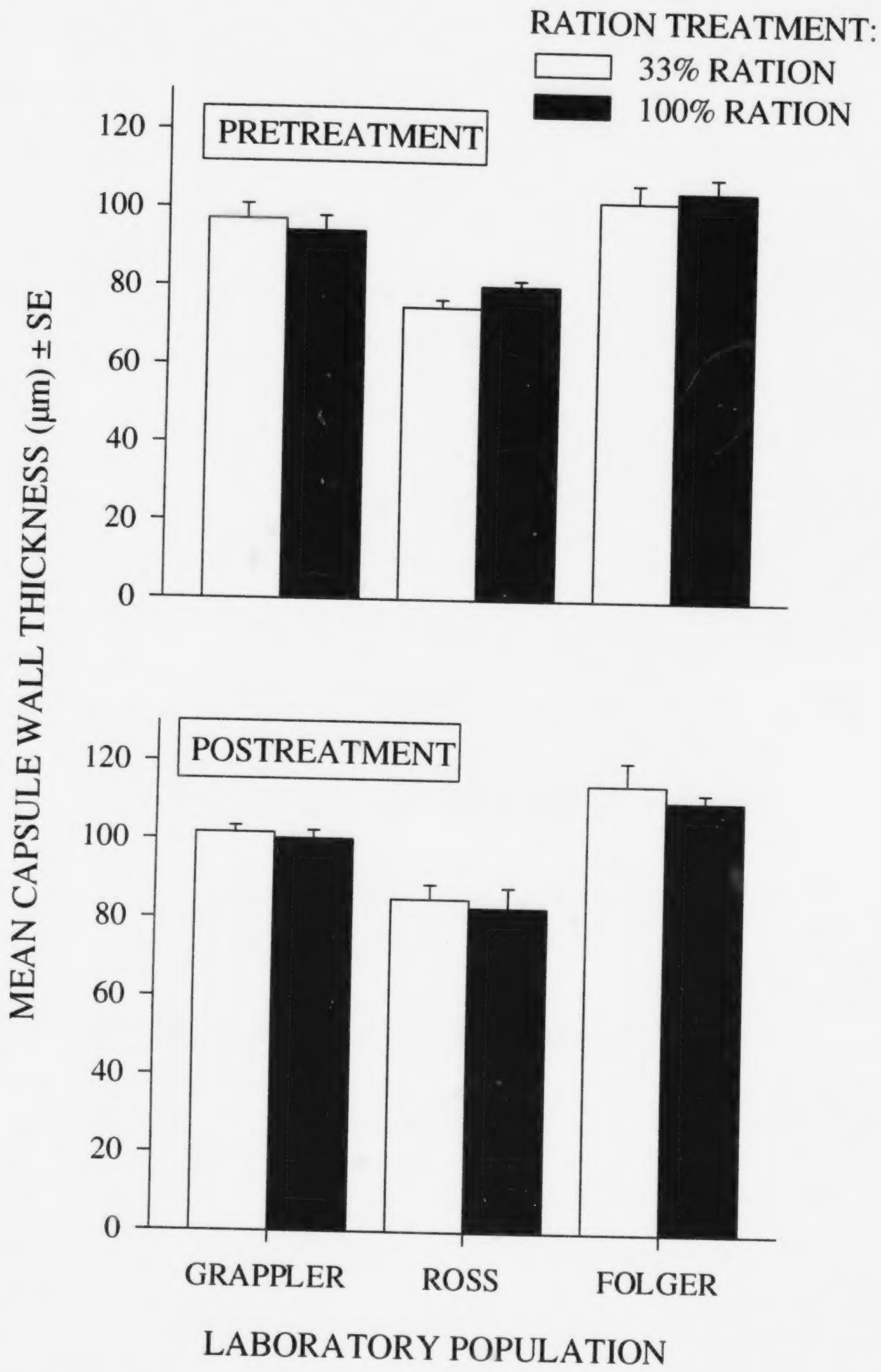

RATION TREATMENT:
33% RATION
100% RATION
PRETREATMENT
POSTREATMENT
MEAN CAPSULE WALL THICKNESS (μm) ± SE
0
20
40
60
80
100
120
GRAPPLER
ROSS
FOLGER
LABORATORY POPULATION

Figure 2-18. Mean number of capsules (± SE) spawned by female *Nucella emarginata* raised from three laboratory (F_1) populations (Grappler Inlet, Ross Islet, and Folger Island) when fed on three different ration treatments: 100%, 66%, and 33% ration. Capsules were collected from each female at monthly intervals and averaged over the nine snails in each treatment group. Three Ross Islet snails died during the study period. The results of an ANOVA comparing the mean number of capsules produced in each treatment group over this year-long period are shown in Table 2-6h.

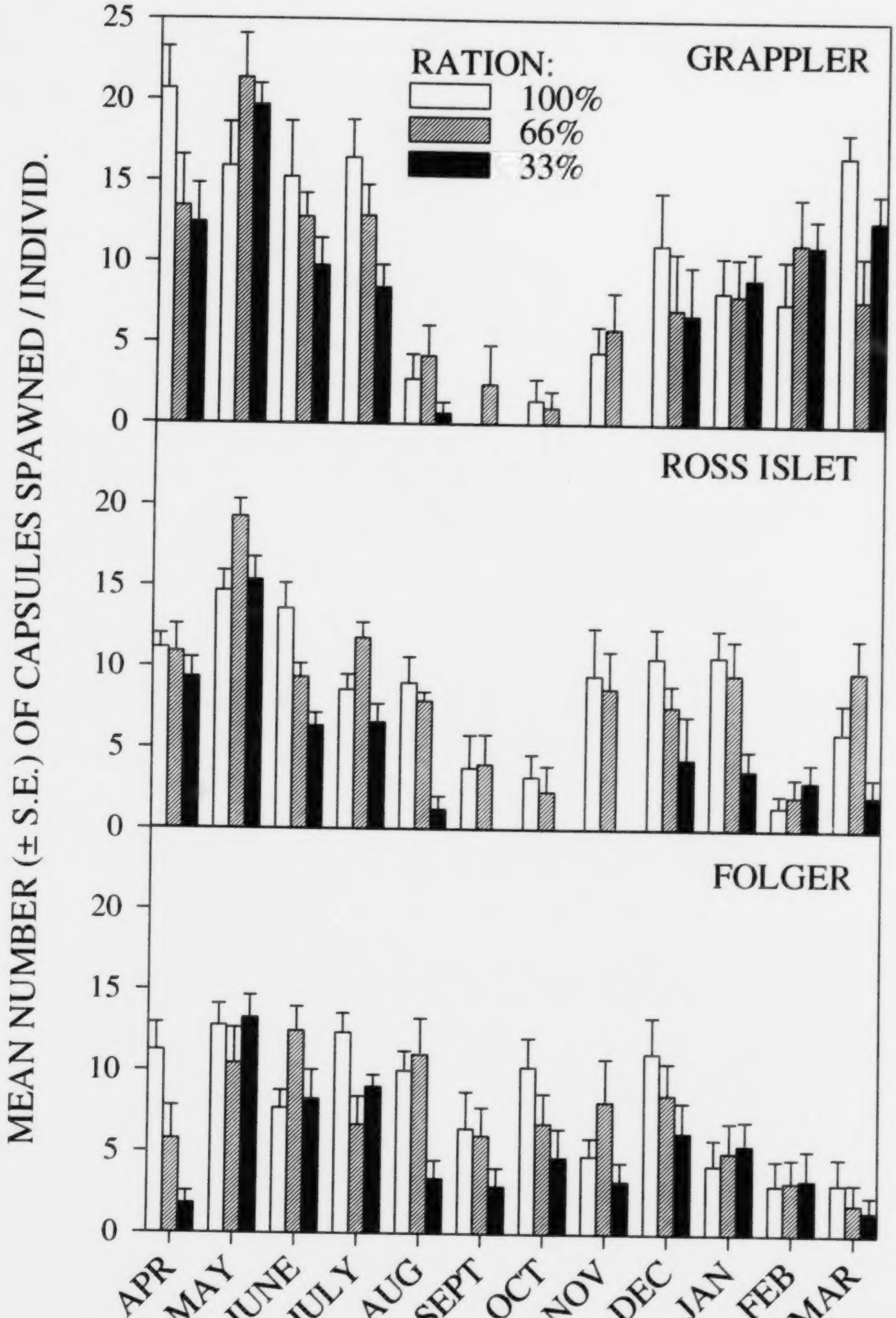
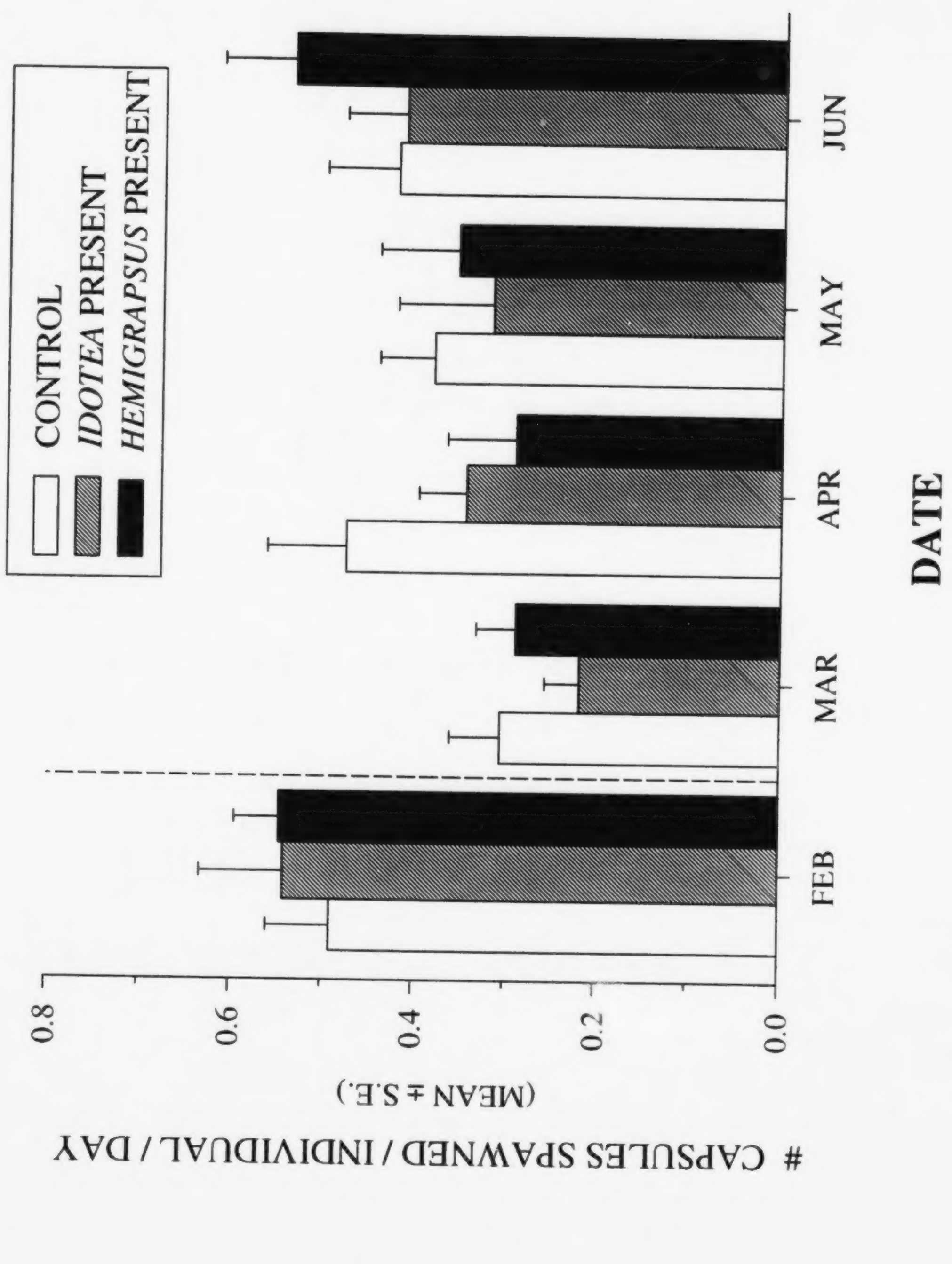

MEAN NUMBER (± S.E.) OF CAPSULES SPAWNED / INDIVID.
RATION:
100%
66%
33%
GRAPPLER
ROSS ISLET
FOLGER
0
5
10
15
20
25
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
JAN
FEB
MAR

Figure 2-19. Capsule production by *Nucella emarginata* individuals before and during exposure to water-borne cues released from *Idotea* feeding on *Nucella* egg capsules, *Hemigrapsus* feeding on *Nucella* egg capsules, or control treatments containing no capsule-eating predators. Twelve female snails were exposed to each treatment condition, four from each of Grappler, Ross and Folger laboratory populations. The vertical dotted line indicates the onset of treatment conditions. A two way ANOVA was used to compare the number of capsules spawned over the treatment period among treatment conditions and laboratory populations. The results indicated a significant difference among laboratory populations (F(Populations) = 5.331, df = 2, 27, $P < 0.011$; population means not shown); but no effect of predator treatment (F(Treatment) = 1.733, df = 2, 27, $P = 0.196$; F(Interaction) = 1.598, df = 4, 27, $P = 0.203$).



# CAPSULES SPAWNED / INDIVIDUAL / DAY
(MEAN ± S.E.)
0.0
0.2
0.4
0.6
0.8
FEB
MAR
APR
MAY
JUN
DATE
CONTROL
IDOTEA PRESENT
HEMIGRAPSUS PRESENT

Figure 2-20. Mean wall thickness (± SE) of capsules produced by *Nucella emarginata* individuals after three months exposure to control conditions or to water-borne cues released by capsule eating predators *Idotea wosnesenskii*, and *Hemigrapsus nudus*. Three capsules were collected per female, and four females were examined per treatment group. A two-way ANOVA comparing differences among treatments and among laboratory populations indicated no significant effect of treatment or source population (F(Treatment) = 0.589, df = 2, 26, P = 0.562; F(Population) = 0.877, df = 2, 26, P = 0.428; F(Interaction) = 0.690, df = 4, 26, P = 0.605.

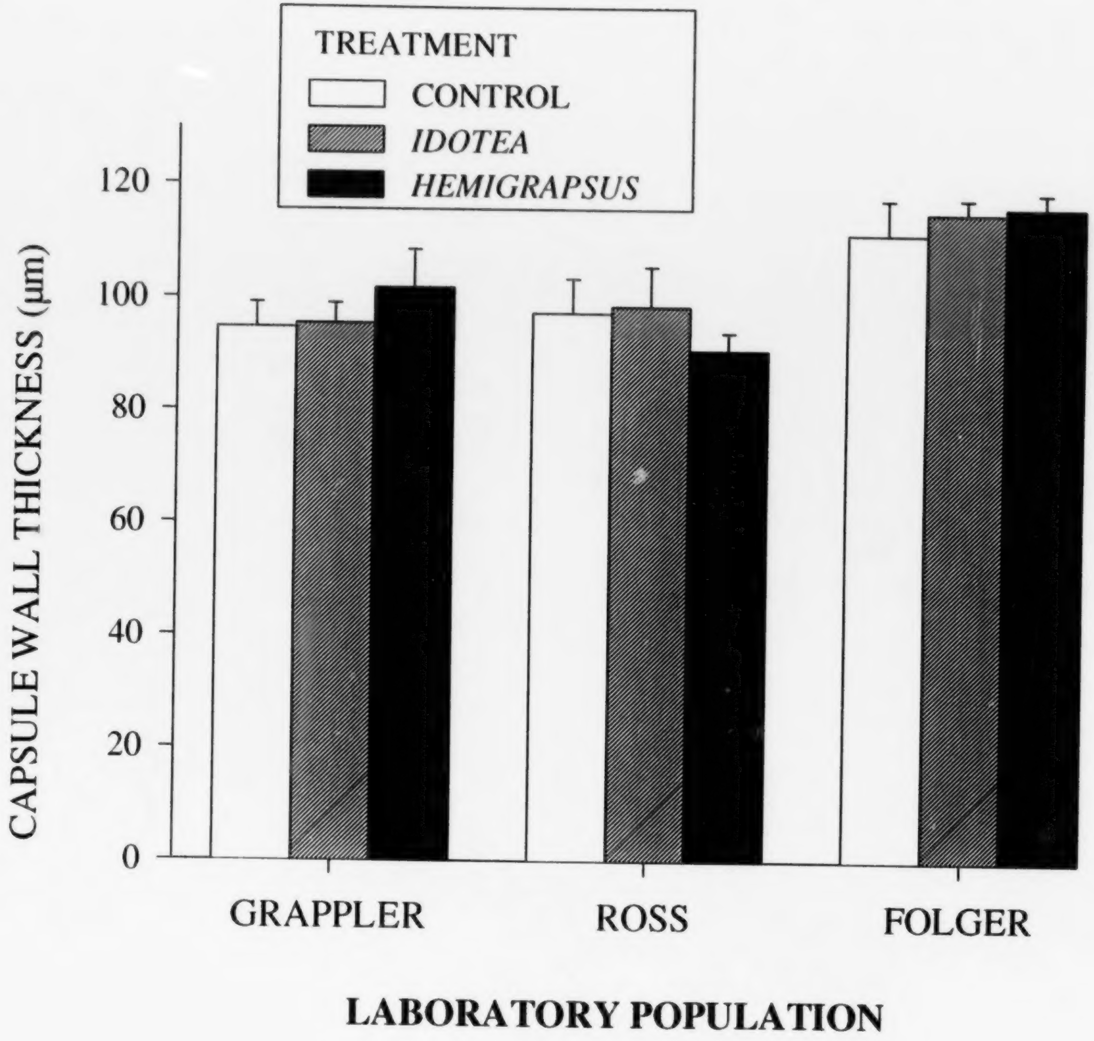
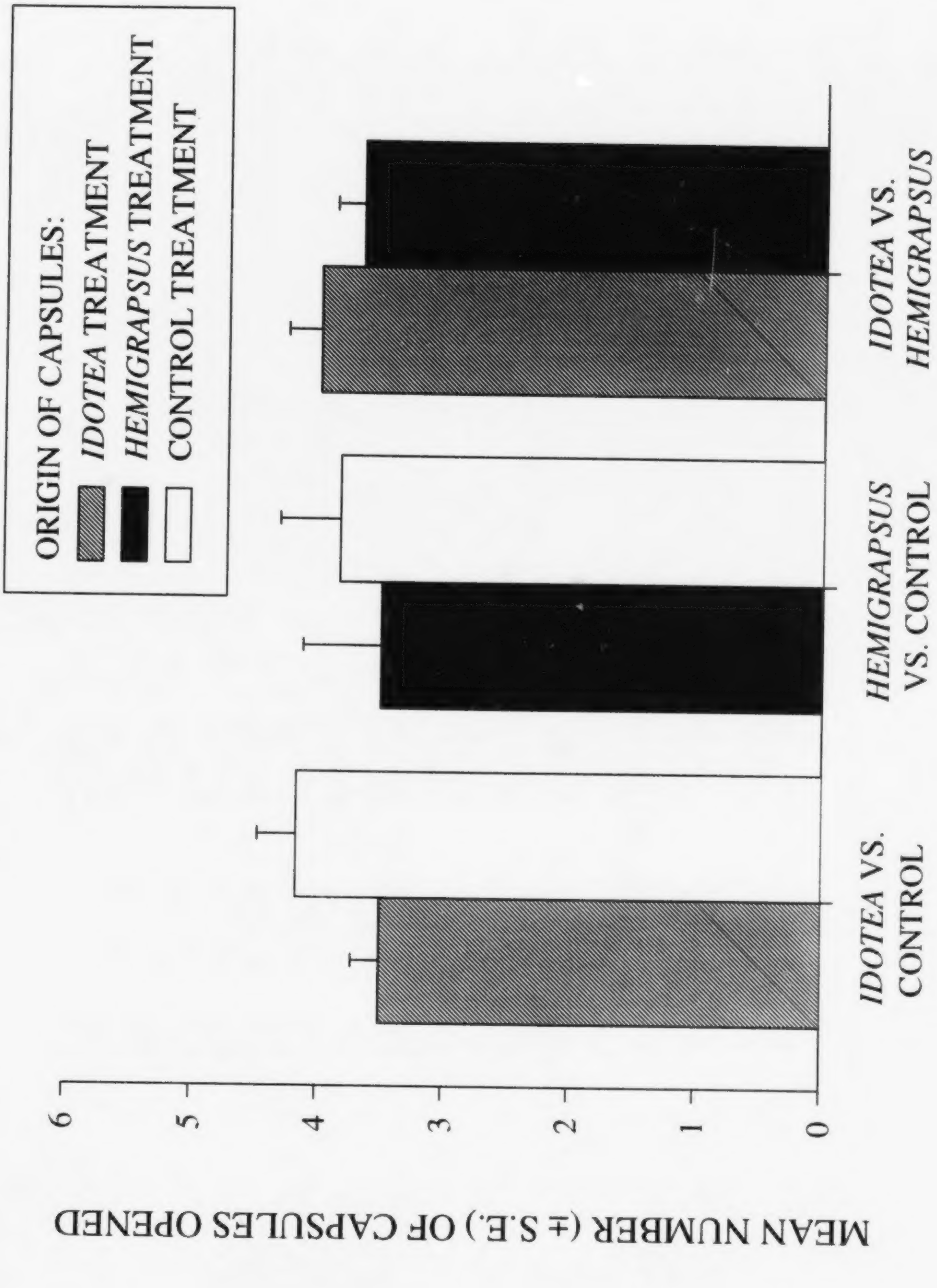

TREATMENT
CONTROL
IDOTEA
HEMIGRAPSUS
CAPSULE WALL THICKNESS (µm)
120
100
80
60
40
20
0
GRAPPLER
ROSS
FOLGER
LABORATORY POPULATION

Figure 2-21. Preference of isopod predators, *Idotea wosnesenskii*, for egg capsules of *Nucella emarginata* spawned under control conditions, or during exposure to water borne cues released by isopods (*Idotea*) and crabs (*Hemigrapsus*) feeding on *Nucella* egg capsules. *Idotea* were presented eight capsules from each of two treatment conditions (n = 16 capsules total) and the first eight capsules opened were recorded (n = 6 replicates per paired treatment comparisons). Contingency table analyses were used to compare the frequency with which capsules from paired treatment conditions were opened by *Idotea* (for data pooled across replicates). These comparisons did not reveal any significant differences in the preference of *Idotea* for capsules produced in any one treatment condition (*Idotea* vs. Control: $G = 0.658$, $P > 0.25$; *Hemigrapsus* vs. Control: $G = 0.165$, $P > 0.50$; *Hemigrapsus* vs. *Idotea*: $G = 0.164$, $P > 0.50$).


ORIGIN OF CAPSULES:
▨ IDOTEA TREATMENT
■ HEMIGRAPSUS TREATMENT
□ CONTROL TREATMENT
MEAN NUMBER (± S.E.) OF CAPSULES OPENED
0
1
2
3
4
5
6
IDOTEA VS. CONTROL
HEMIGRAPSUS VS. CONTROL
IDOTEA VS. HEMIGRAPSUS

CHAPTER 3

Encapsulation of eggs by marine gastropods: effect of variation in capsule form on the vulnerability of embryos to predation[1]

Abstract

Representatives of many plant and animal taxa enclose their embryos within some form of protective structure. Inter- and intraspecific differences in the morphology of these egg coverings may have profound effects on the development and survival of encapsulated embryos, yet in many taxa little is known about the causes or potential consequences of this variation. Comparisons of capsule morphology among populations of the rocky shore gastropod, *Nucella emarginata*, revealed significant variation in the thickness of capsule walls, the only barrier separating developing embryos from the external environment. Laboratory experiments demonstrated that thicker walled capsules were more resistant to predation by a co-occurring isopod, *Gnorimosphaeroma oregonense*, than were thinner walled capsules. Control experiments confirmed that these differences in vulnerability were not due to differences in the palatability of the capsule wall or attractiveness of the capsule contents. The actual mechanism by which thick walled capsules differentially protect developing embryos remains unclear, although decreased vulnerability of thick walled capsules to these isopods may simply result from increased handling time by predators. Subtle differences in capsule morphology thus appear to have substantial effects on the survival of encapsulated embryos. Hence, predators may have played an important role in selecting for thick-walled capsules among populations of *Nucella emarginata*.

Introduction

Maternally-derived egg coverings, such as egg shells and egg capsules, have had a major evolutionary impact on the reproductive success and habitat expansion of many taxonomic groups. In terrestrial environments, for instance, seed pods and shelled amniotic eggs have allowed plants and vertebrates to exploit new habitats by reducing their dependence on moist conditions for reproduction (Little, 1983; Stewart, 1983). Likewise, structurally-complex egg shells resistant to mechanical damage, predation, desiccation, and immersion (Hinton, 1981; Margaritis, 1985), are believed to have contributed to the extraordinary success of insects, relative to other terrestrial arthropods, and may have provided the potential for the rapid and extensive diversification of the Insecta (Zeh et al., 1989). In marine environments, benthic egg capsules and gelatinous egg masses have also been advantageous to such organisms as flatworms, annelids, molluscs, and chordates, by providing developing embryos with a refuge from sources of mortality associated with a planktonic existence (Pechenik, 1979; Strathmann, 1985; Rumrill, 1990). The encapsulation of eggs within protective structures, therefore, appears to have evolved independently in a number of phylogenetically diverse groups in response to the varied risks associated with embryonic development.

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[1] A version of this chapter has been published: Rawlings, T.A. 1994. Encapsulation of eggs by marine gastropods: Effect of variation in capsule form on the vulnerability of embryos to predation. Evolution 48: 1301-1313.

Although encapsulation is a widespread phenomenon within both plant and animal phyla, little is known about the precise morphological and physiological consequences of egg coverings. This is surprising in view of the considerable variation that exists in the morphology of encapsulating structures. Marine prosobranch gastropods, for instance, deposit embryos within a tremendous variety of structures ranging from gelatinous masses to tough, structurally-complex capsules (Fretter, 1984). The form of these structures can vary dramatically within families (Thorson, 1935; D'Asaro, 1970; 1988; 1991; Robertson, 1974; Strathmann, 1987), among closely-related species (Ostergaard, 1950; Perron, 1981; Perron and Corpuz, 1982; Palmer et al., 1990; Reid, 1990; Collins et al., in review) and even among populations of a single species (Rawlings, 1990). Although such inter- and intraspecific differences may have significant implications for embryonic development and survival, the causes and potential consequences of this variation remain largely unknown (see Pechenik, 1986).

Variation in the morphology of encapsulating structures may reflect the outcome of different selective pressures acting upon embryos during their development. Gastropod egg capsules reduce the vulnerability of developing embryos to sources of mortality such as predation (Spight, 1977; Perron, 1981; Rawlings, 1990), salinity stress (Pechenik, 1982; 1983; Hawkins and Hutchinson, 1988), desiccation (Spight, 1977; Pechenik, 1978; Chapter 4), bacterial attack (Pechenik et al., 1984; Lord, 1986) and possibly ultraviolet light (Chapter 5). Hence, differences in capsule form may result from adaptive responses to these sources of mortality.

Predation may have played an important role in the evolution of capsule morphology. As developing eggs represent an attractive food source (Orians and Janzen, 1974), and the development of encapsulated prosobranch embryos can last as long as eight months in some species (e.g. West, 1973), predation on egg capsules can be severe (MacKenzie, 1961; Emlen, 1966; Phillips, 1969; West, 1973; Brenchley, 1982; Race, 1982; Abe, 1983; Martel et al., 1986; Rawlings, 1990). In response, many prosobranch gastropods have evolved mechanisms that appear to reduce predation on encapsulated embryos. Some, for instance, actively defend capsules from predators (Ostergaard, 1950; D'Asaro, 1970; Eaton, 1971). Others brood capsules in the protective confines of the mantle cavity, foot, or shell umbilicus (D'Asaro, 1970; Robertson, 1970; Hoagland, 1986), or deposit them next to protective babysitters (Shimek, 1981). Among many of the higher caenogastropods, however, encapsulated embryos are not protected by the parent. In these species, the structural properties of the capsular case itself represent the primary defense.

Although some gastropod egg capsules may deter predators more effectively than others, variability in the resistance of capsules to predators has never been examined specifically. Wall strength and the proportion of reproductive energy invested in capsular cases of *Conus* species increase with the development time of encapsulated embryos (Perron, 1981). Hence, embryos with more protracted intracapsular development are enclosed within stronger and energetically more expensive capsules than those with shorter development, possibly as a direct consequence of differential exposure to predators (Perron, 1981; Perron and Corpuz, 1982). Capsule wall thickness also varies intraspecifically in association with specific egg capsule predators (Rawlings, 1990), suggesting that among-population differences in capsule morphology may result from differing intensities of predation. However, other explanations are possible. Direct tests of the resistance of different capsule morphologies to predation are necessary, therefore, if the functional significance of capsule form is to be assessed.

In the present study, I examined the effect of variation in wall thickness on the resistance of benthic egg capsules of the marine intertidal snail, *Nucella emarginata* (Deshayes, 1839; [northern species; Palmer et al., 1990]) (Prosobranchia: Muricidae), to predation by the isopod *Gnorimosphaeroma oregonense* (Dana, 1852). Wall thickness varies among populations of *Nucella emarginata* (Rawlings, 1990). As the capsule wall represents the only physical barrier separating developing embryos from their external environment, and these snails exhibit no form of parental defense of encapsulated embryos, capsule wall thickness may indicate the potential resistance of capsular cases to specific environmental risks.

Materials and Methods

Intraspecific variation in capsule wall thickness

To determine the extent of variation in capsule wall thickness among local populations of the marine intertidal snail, *Nucella emarginata*, in May 1990 I collected ten clutches of capsules from each of eight snail populations near the Bamfield Marine Station, Barkley Sound, British Columbia (48°50'N; 125°08'W). Collection sites were geographically separated along a gradient of wave-exposure; these have been ranked tentatively in wave exposure based on 1) the maximum height of the *Balanus glandula* (Darwin, 1854) zone, and 2) the lowest height of vascular plants (Palmer et al., unpub. data). Increased wave exposure is generally correlated with an enhanced upward extension of the *B. glandula* zone (Kitching, 1976; Carefoot, 1977) and a reduced downward extension of the lowest vascular plants (Palmer et al., unpub. data).

To compare capsule wall thickness among populations, one capsule was selected from each of ten clutches for each population. Capsules were measured, marked at a point 70% along the length of the chamber housing developing embryos, and then sectioned at the marked point using a freeze microtome. Capsule walls appear to be thinnest and least variable in this region (see Rawlings, 1990). Capsule sections were mounted under a compound microscope equipped with an ocular micrometer. Wall thickness was determined by taking the average of eight measurements recorded around the circumference of the mounted section, using the two seams of the capsule wall as reference points.

Susceptibility of thick- and thin-walled egg capsules to *G. oregonense*

The marine intertidal isopod *Gnorimosphaeroma oregonense* was chosen as the capsule-eating predator in this study. Isopods are known to be important predators on gastropod egg capsules in the field (Emlen, 1966; Phillips, 1969; Spight, 1977; Rawlings, 1990), and feed on encapsulated embryos by gradually abrading through the capsule wall until the capsule chamber is ruptured (Rawlings, pers. obs.). Although *G. oregonense* individuals have not been reported to open *Nucella* egg capsules previously, they are common inhabitants of rocky intertidal communities (Kozloff, 1973), can be found associated with capsule masses, and have often been observed feeding on encapsulated embryos in the field (Rawlings, pers. obs.). The small size (< 1 cm in body length) and high density (mean densities > 2000 m^{-2}; Brook et al., 1994) of these isopods, make them representative of other small crustacean predators that may frequent capsule masses during embryonic development.

To compare the relative resistance of different capsule morphologies to these predators, thick- and thin-walled capsules were offered to *Gnorimosphaeroma oregonense* individuals in two different experiments. For Experiment I, isopods were obtained from Grappler Inlet (48°49'54"N; 125°06'54"W) in April 1992 by collecting small barnacle-covered rocks from a low intertidal channel (1.6 m above extreme low water, spring [ELWS] (Canadian Datum). Once in the laboratory, isopods were removed from the rocks, measured for total body length at 12X magnification using a dissecting microscope equipped with an ocular micrometer (mean ± S.E: 6.9 ± 0.24 mm; n = 35), and then individually placed in labeled mesh-paneled cages ("microfuge cage"; Fig. 3-1a). Within a cage, each isopod was given two egg capsules upon which to feed: a thick-walled capsule from Kirby Point (48°50'42" N; 125°12'24" W) and a thinner-walled capsule from Ross Islet (48°52'12" N; 125°06'54" W). Source populations for thick- and thin-walled capsules were chosen based on comparisons of wall thickness differences among sites (see Results) and the local availability of capsules at the time these experiments were conducted. To identify thick- and thin-walled capsules during the course of the experiment, capsules were marked with small colored dots on the plug and base of the capsule chamber using permanent Lumocolor™ pens. The use of specific colors was alternated between capsule morphs to minimize the chance that colour preferences would bias the results. Cages were then placed in a seawater table provided with flowing seawater (11-12°C) and capsules were examined at 2-3 d intervals for evidence of predation. A capsule was considered to have been preyed upon when the capsule chamber containing developing embryos was ruptured. This experiment was stopped after 30 d.

To determine if preferences for thick and thin-walled capsules differed with size and sex of the predator, in June 1992 I repeated this experiment (Experiment II) using larger sample sizes and isopods of known sex. Isopods were freshly collected from Grappler Inlet, as described above, measured, and then sexed. Females were identified by the presence of small or large oostegites, whereas males were recognized by the presence of penes or stylets. Individuals possessing both male and female sexual characteristics (penes and oostegites), indicating that these were in the process of undergoing a protogynous sex change (Brook et al., 1994), were not used in this experiment. The first 40 male and female isopods collected in this manner were individually placed in a microfuge cage, as described above, and then allocated one thin-walled capsule from Ross Islet and one thick-walled from either Grappler Inlet or Kirby Point. Thick-walled capsules were collected from two source populations in this experiment to control for the effects of capsule size, since capsule length varied among sites (mean capsule body length ± S.E. [n=10]: Grappler: 8.9 ± 0.32 mm > Ross: 6.7 ± 0.15 mm > Kirby: 5.9 ± 0.10 mm). In total, 40 isopods (20 male; 20 female) were offered: 1) thin-walled Ross Islet capsules versus larger thick-walled capsules from Grappler Inlet, and 2) thin-walled Ross Islet capsules versus smaller thick-walled capsules from Kirby Point.

Cages were placed in a seawater table provided with fresh seawater (11-12°C) and were examined at 2-4 d intervals to determine if capsules had been opened. The experiment was terminated after 50 d for all cages except those in which only one capsule had been opened. These cages continued to be regularly monitored until the remaining capsule was either opened or embryos within the capsule hatched (day 70).

Palatability of isolated pieces of thick- and thin-walled capsules

To determine if differences in the rates of predation on thick- and thin-walled capsules reflected differences in the palatability of the capsule wall, I offered individual isopods the choice between feeding on pieces (< 2.2 mg, mean = 1.7 mg) of capsular material from thick- and thin-walled capsules. Rings of capsule tissue were removed from 25 capsules collected from Ross Islet (thin-walled) and Kirby Point (thick-walled) by taking transverse sections of the capsule chamber. Care was taken to remove any embryos, nurse eggs, and intracapsular fluid adhering to the capsule pieces. Capsule pieces were then damp-dried with absorbent tissue and weighed to the nearest 0.01 mg. Thick and thin-walled pieces were matched as closely as possible with respect to initial weight (mean differences [± S.E.] = 4.0 ± 0.6%; n = 25) and were then allocated to each of 25 microfuge cages. Capsule rings were identified by stringing a small loop of coloured polyester thread through each ring. Each loop of thread was removed before weighing capsule pieces, so that the thread did not interfere with measurements of capsule weight.

In July 1992, 20 female isopods (mean body length ± S.E.: 5.4 ± 0.18mm) freshly collected from Grappler Inlet were measured and individually placed into cages containing capsule pieces. Five cages, with paired capsule pieces, were left without isopods to control for weight changes due to deterioration of the capsule pieces by micro-organisms or fouling by diatoms. All cages were placed in a seawater table with running seawater at 11-12°C. Capsule pieces were weighed at 7-10 d intervals over a period of 50 d. The amount of material removed from thick and thin-walled pieces was compared per cage as soon as one capsule piece had been entirely consumed within a cage, or on the final day that pieces were weighed (day 50).

Predation on whole versus stripped capsules

If capsule wall thickness influences the susceptibility of encapsulated embryos to *Gnorimosphaeroma*, then embryos lacking such a barrier should be subject to more intense predation than those within whole capsules. I examined this by experimentally stripping capsules of portions of their outer wall (laminae 1 and 2; Chapter 6; Rawlings, 1995) leaving embryos protected by a thin inner wall (lamina 3) only a few microns thick. The resistance of these capsules to isopod predators was then compared relative to whole capsules.

In September 1992, 50 capsules were collected intertidally from Kirby Point and then randomly divided into two groups. In the first group, approximately one third of the outer capsule wall was stripped away from capsules using a microtome blade (hereafter termed "stripped" capsules). Care was taken to avoid puncturing the underlying lamina. Capsules in the second group were left intact (hereafter termed "whole" capsules). One stripped and one whole capsule were allocated to each of 25 large mesh-panelled cages ("minifuge cage"; Fig. 3-1b). Because of the delicate nature of stripped capsules, each capsule was attached to a holdfast within the cage made from Tygon™ tubing. Capsule stalks were inserted into small slits perpendicular to the length of the tubing and thus were held in a fixed position throughout (Fig. 3-1b). Female isopods (mean length ± S.E.: 5.6 ± 0.15 mm, n = 15), freshly collected from Grappler Inlet, were allocated to 15 minifuge cages. Ten cages were left without isopods to compare the survivorship of embryos within stripped and whole capsules when not exposed to isopod predators. Capsules were checked daily for a period of 20 d to determine 1) whether the capsule chamber had been ruptured, as indicated by fluid leaking from the capsule chamber, and 2) if the contents had been entirely consumed, as determined by the absence of nurse eggs and embryos within the capsule chamber.

Isopod preferences for stripped capsules from two different populations

Because capsules of different wall thickness were taken from different populations, predator preferences for one capsule morph over another could either reflect variation in the protective quality of the capsular case itself or in the attractiveness of the eggs and fluid within the capsule. To determine if isopods preferred the capsular contents of one capsule morph over another, I offered *Gnorimosphaeroma oregonense* individuals the choice of experimentally stripped egg capsules collected from Ross Islet (originally thin-walled) and Kirby Point (originally thick-walled). Forty capsules were collected from Kirby Point and Ross Islet in October/November 1992. Capsules from each population were marked by tying a loop of colored polyester thread around the stalk and were then stripped of approximately one third of their outer capsule wall. One stripped capsule from each source population was placed into a Tygon™ holder and allocated to a minifuge cage. Freshly collected female isopods (mean length ± S.E.: 5.4 ± 0.06 mm, n = 30) were individually placed in 30 cages, while 10 cages were left without isopods. Capsules were checked daily and the first capsule ruptured and the first capsule emptied were recorded per cage.

Results

Intraspecific variation in capsule wall thickness

The thickness of capsule walls differed significantly among populations of *Nucella emarginata* (ANOVA: $F_{(7,72)} = 12.5$, $P < 0.0001$; Fig. 3-2[2]). Capsule walls were thickest at the most wave-sheltered and wave-exposed sites, and thinnest at sites experiencing intermediate wave action. Wall thickness measurements from Grappler Inlet (mean ± S.E. = 83.5 ± 2.3 μm, n = 10) and Ross Islet (55.7 ± 1.7 μm, n = 10) were similar to those from capsules collected from the same study sites in March 1988 (means of 80.8 ± 2.2 μm, n = 8, and 60.6 ± 2.6 μm, n = 8, respectively; Rawlings, 1990), suggesting that site-differences in capsule wall thickness remain relatively constant over time.

Susceptibility of thick- and thin-walled egg capsules to G. oregonense

A large body size did not confer an obvious advantage to isopods in terms of predation rate on *Nucella* egg capsules. Despite considerable sexual dimorphism in the body size of male and female *Gnorimosphaeroma oregonense*, with female isopods having significantly smaller body lengths than males (mean length ± S.E. = 5.8 ± 0.09 mm, n = 40, versus 8.1 ± 0.08 mm, n = 40, respectively; ANOVA: $F = 360$, $P < 0.0001$)), only 28% of surviving males (n = 32) opened capsules compared to 63% of surviving females (n = 40) over a 50 day period. Within each sex, body length was not associated with the time taken to open the first capsule within a cage (males: $r^2 = 0.003$, $P > 0.25$, n = 9; females: $r^2 = 0.031$, $P > 0.25$, n = 25). Among sexes, male isopods did not open their first capsule significantly faster than females (means ± S.E. = 22.7 ± 4.6 [n = 9] and 25.4 ± 3.1 days [n = 25], respectively; ANOVA: $F = 0.35$, $P > 0.5$).

Both preference experiments (Experiment I; Experiment II) indicated that *Gnorimosphaeroma oregonense* individuals opened thin-walled egg capsules from Ross Islet before thick-walled capsules from either Grappler Inlet or Kirby Point (Table 3-1;

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[2] Site rankings along this wave-exposure gradient differ slightly from those in Chapter 2 because measurements of wave amplitude (an additional variable for ranking sites) were not yet available for all sites.

Fig. 3-3). This trend was exhibited by both male and female isopods (Table 3-1). Thin-walled capsules were opened first in 15 out of 17 cages in which capsules were opened over a 30 day period (Experiment I), and in 30 out of the 34 cages over a 50 day period (Experiment II). Despite this preference, isopods nibbled both capsule morphs prior to rupturing the capsule chamber. Once capsules had been opened, generally near the base of the capsule chamber, the contents were readily consumed within a few days.

Thick-walled capsules were not impervious to isopods. In Experiment II, both thick- and thin-walled capsules were eventually opened in 23 cages (closed symbols, Fig. 3-4). Assuming an equal rate of grazing on thick- and thin-walled capsules, and no difference in the time taken to penetrate these capsule morphs, plots of the time taken to open each capsule within a cage would be expected to fall along the diagonal line illustrated in Figure 4. Instead, the majority of symbols fell above the line, thus indicating that thick-walled capsules took longer to be opened. This difference was significant, with means (± S.E.) of 23.3 ± 3.1 days and 41.1 ± 3.8 days to open thin- and thick-walled capsules, respectively (Wilcoxon matched-pairs signed-ranks test: $t = 12.5$, $P < 0.001$, $n = 23$).

Palatability of isolated pieces of thick- and thin-walled capsules

Gnorimosphaeroma oregonense individuals consumed relatively little egg capsule material over 50 days. On average, isopods ate only 24% of the capsule material offered to them, and only one capsule piece was completely consumed during this period. Nevertheless, capsule pieces exposed to isopods lost more weight relative to control pieces (Fig. 3-5). Comparisons of the amount of tissue consumed from thick- and thin-walled capsule pieces indicated that isopods did not exhibit a significant preference for either type of material (Paired t-test: $t = 0.55$, $P > 0.5$, $n = 20$).

Predation on whole versus stripped capsules

Nucella egg capsules stripped of part of their outer wall were significantly more susceptible to isopod predators than whole capsules (Fig. 3-6). Over a period of 20 days, 13 of 15 stripped capsules were ruptured by isopods, whereas no whole capsules were opened during this time interval (Fig. 3-6; Chi-square test: $\chi = 7.11$, $P < 0.01$). Of the ruptured stripped capsules, nine were subsequently emptied of all contents, while contents were only partially consumed in the remaining four capsules. Stripped capsules were also more vulnerable to attack by micro-organisms. In control cages, protists entered two of 10 stripped capsules by breaking through the inner lamina into the capsule chamber. Embryos within these capsules died shortly thereafter. No whole capsules became infested with protists.

Isopod preferences for stripped capsules from two different populations

Gnorimosphaeroma oregonense individuals did not show any preference for rupturing or emptying Ross Islet (originally thin-walled) or Kirby Point capsules (originally thick-walled) once a portion of the outer capsule wall of these capsules had been stripped away (Table 3-2). In all cages but one, isopods entered the capsule chamber through the experimentally stripped portion of the capsular case, rather than through the remaining intact portion. Hence, *G. oregonense* individuals preferentially opened capsules through the thinnest portion of the capsule wall. Predation rates on stripped capsules were reasonably fast compared to previous experiments using whole capsules (see Fig. 3-3). At least one capsule was ruptured per cage by day 7 (n = 30) and emptied by day 20 (n = 27). Sixteen of the 20 control capsules remained intact over the 20 day period, while four became infested with protists.

Discussion

Evolution of egg coverings within the Gastropoda

Despite the tremendous variety of egg capsules and masses produced by gastropods, little is known about the evolution of egg coverings within this group. Encapsulation is undoubtedly a derived trait, since maternally derived egg coverings are rarely found in patellogastropods (Lindberg, 1988) and lower archaeogastropods (Fretter, 1984; Hadfield and Strathmann, 1990), yet gastropod phylogeny still remains too coarsely resolved to determine how many times encapsulation may have evolved (Bieler, 1992). Based on current phylogenies of the Gastropoda (see Haszprunar, 1988; Lindberg and Ponder, 1991; Tillier et al., 1992), however, encapsulation appears to have arisen independently in at least two clades. Vetigastropods, one subclade of the Archaeogastropoda (Lindberg and Ponder, 1991), are typically viewed as gastropods with simple reproductive systems bearing no glandular structures for encapsulating eggs. Nevertheless, some higher vetigastropods, such as trochoidean snails, encapsulate their eggs in envelopes and gelatinous coverings that are derived from the ovary and urogenital papilla (Fretter, 1984; Hadfield and Strathmann, 1990). Since vetigastropods are the only gastropods to produce protective egg coverings from the ovary (Fretter, 1984), and these snails are not believed to share a common capsule-laying ancestor with the caenogastropods (although see Hadfield and Strathmann, 1990), this suggests that encapsulation has evolved independently in these two groups. The complex reproductive system, glandular oviduct, and protective egg coverings of certain neomphaloid limpets (Fretter et al., 1981; McLean, 1981), another subclade within the Archaeogastropoda, also suggest a different origin of encapsulation from the Caenogastropoda. Given that encapsulation has evolved more than once within the Gastropoda, therefore, egg coverings seem likely to be adaptive.

Adaptive significance of variation in the morphology of encapsulating structures

If encapsulation has evolved within the Gastropoda as an adaptive response to risks experienced by embryos during their development (Pechenik, 1979; Strathmann, 1985; Rumrill, 1990), variation in morphology of egg coverings may reflect an adaptive response to different selective pressures within the varied habitats in which capsules are deposited or released. Some anecdotal evidence supports this. The incorporation of calcium into benthic egg capsules and egg shells of various neritimorph, caenogastropod and pulmonate snails, for instance, is associated with the independent movement of these groups into terrestrial environments (Tompa, 1980). Likewise, the egg shells of pulmonate gastropods and terrestrial vertebrates have converged in form presumably as an adaptive response to a common terrestrial habitat (Tompa, 1984). Even within similar habitats, however, gastropods produce a tremendous variety of encapsulating structures. Although differences in phylogenetic history may explain some of this variation, capsule form can vary substantially even among closely related species living in similar habitats (Buckland-Nicks et al., 1973; Perron, 1981; Palmer et al., 1990; Collins et al., in review). Clearly, therefore, much remains to be understood about the adaptive significance of differences in capsule form.

Cladistic analyses of relationships within and among gastropod families should ultimately help to elucidate the adaptive significance of differences in the form of encapsulating structures (see Reid, 1990). The joint mapping of spawn characteristics and life-history or ecological attributes onto independently-derived cladograms, for instance, could enable a formal comparative test (sensu Harvey and Pagel, 1991) of adaptive explanations. Although such an approach could provide considerable information on the evolution of encapsulating structures within the Gastropoda, it depends critically on a rigorously constructed phylogeny.

Intraspecific variation: a tool for assessing the adaptive significance of differences in capsule morphology.

Intraspecific variation in capsule morphology can also provide a key to understanding the adaptive significance of differences in capsule form (Rawlings, 1990). Variation in capsule morphology among populations can be used to assess the benefits and costs of differences in capsule form without the potentially confounding phylogenetic effects associated with interspecific comparisons. Therefore, if differences in capsule morphology are correlated with specific selective pressures in the field, and if such differences can be shown experimentally to affect the vulnerability of embryos to these stresses, an adaptive explanation seems likely.

Intraspecific differences in capsule morphology do indeed have a significant effect on the susceptibility of encapsulated embryos to specific capsule-eating predators in the system examined here. The intertidal isopod, *Gnorimosphaeroma oregonense*, opened significantly more thin-walled capsules of *Nucella emarginata* when given a choice between thick- and thin-walled morphs (Fig. 3-3, Table 3-1). *Idotea wosnesenskii*, another intertidal isopod, exhibits a similar preference for thin-walled capsules (Rawlings, 1990). Differences in the vulnerability of *Nucella* capsules to isopod predators may be very significant ecologically. Although predation rates by *G. oregonense* were low in this study relative to previous laboratory experiments (Rawlings, unpub. data), possibly reflecting seasonal differences in predation intensity, isopods are undoubtedly one of the most important predators on *Nucella* egg capsules in the field (Emlen, 1966; Spight, 1977; Rawlings, 1990). A one-time census at Grappler Inlet in May 1988, found isopod bite-marks associated with 43 - 100 % of *N. emarginata* capsules ruptured by predators (Rawlings, 1990). As *G. oregonense* individuals are often present in densities averaging over 2000 m^{-2} (Brook et al., 1994), and feed on gastropod egg capsules in the field (Rawlings, pers. obs), these isopods have the potential to represent a serious threat to encapsulated *N. emarginata* embryos.

Differential susceptibility of encapsulated *Nucella* embryos to predation by *Gnorimosphaeroma oregonense* individuals appears to reflect differences in the thickness of the capsule wall rather than the palatability of the capsule material or the attractiveness of the capsule contents. Isopods did not show any preference for pieces of capsule material from thick versus thin-walled capsules (Fig. 3-5). In fact, little capsular material was consumed from either type of capsule over 50 d when embryos were absent. Although predators can digest the capsule walls of some gastropod egg capsules (*Ilyanassa obsoleta*; Brenchley, 1982), intertidal crabs and isopods rarely eat large quantities of the capsular case of *Nucella* capsules and appear unable to digest this material once consumed (Rawlings, 1989). Hence, it is unlikely that predators derive any nutrition from the capsule wall itself.

Gnorimosphaeroma oregonense individuals also did not differentiate between the contents of thick- and thin-walled capsules. Once wall thickness differences were eliminated by stripping away portions of the capsular case, encapsulated embryos from both thick- and thin-walled morphs were equally susceptible to predation (Table 3-2). Capsule wall thickness thus appears to be the primary factor determining the susceptibility of encapsulated embryos to isopod predators.

The actual mechanism by which thick-walled capsules differentially protect embryos from isopod predators remains unclear. *Gnorimosphaeroma oregonense* individuals gradually abrade through the capsule wall, usually near the base of the capsule chamber, until the chamber is penetrated. Since isopods chewed on both thick- and thin-walled capsules prior to opening one capsule, the higher consumption rate of thin-walled capsules may simply have resulted from shorter handling times. The rate of predation on *N. emarginata* capsules also increased dramatically when portions of the outer capsule wall were removed (Fig. 3-6), again suggesting that wall thickness may be a limiting factor. Alternatively, however, isopods may actively choose between thick- and thin-walled capsules based on other stimuli associated with wall thickness. Differences in wall thickness, for instance, may be reflected in the amount of stimulus released per unit area of thick- and thin-walled capsules, such that thinner walled capsules are more attractive to predators. Clearly, more work is necessary to determine whether isopods actively discriminate between thick- versus thin-walled capsules.

Since many organisms are able to feed on prosobranch egg capsules, thicker-walled capsules are unlikely to be more resistant to all such predators. Capsular cases have been opened in the laboratory and field by such predators as: decapods (MacKenzie, 1961; Spight, 1977; Perron, 1981; Brenchley, 1982; Rawlings, 1990), isopods (Emlen, 1966; Phillips, 1969; Spight, 1977; Rawlings, 1990), polychaetes (Feare, 1970), chitons (Emlen, 1966; Eaton, 1971; Rawlings, 1990), prosobranch gastropods (Phillips, 1969; Eaton, 1971; West, 1973; McKillup and Butler, 1979; Brenchley, 1982; Race, 1982, Abe, 1983), and echinoderms (Eaton, 1971; Spight, 1977; Martel et al., 1986). Preliminary experiments have revealed that the intertidal shore crabs, *Hemigrapsus spp.*, exhibit no preference for thin- versus thick-walled capsules (Rawlings, unpub. data). These crabs open capsules by chewing through the capsular plug with their mandibles or rupturing the capsule chamber in their chelipeds (Rawlings, 1990). Differences in wall thickness, therefore, may be insufficient to affect the susceptibility of embryos to this type of predation. Nevertheless, even if some predators can consume any form of capsule, thick-walled capsules may still be adaptive if they are effective against an important subset of predators. Since thin-walled capsules are more vulnerable to isopods (Rawlings, 1990; this study) and these are the dominant predators on *N. emarginata* egg capsules at some locations (Rawlings, 1990), isopods may be a selective agent favoring the production of thick capsule walls.

The intensity of predation varies along gradients of wave-exposure, with lower rates of predation generally occurring on more wave-exposed shores (e.g. Kitching et al., 1959; Menge, 1978a; 1978b). Interestingly, capsule wall thickness in *Nucella emarginata* did not reflect these predicted site-differences in predation intensity. This should not necessarily be expected, however. Foraging rates of predators can be affected by wave-action (Menge, 1978a; Menge, 1978b), yet the type of capsule-eating predators may vary independently of wave-exposure (Rawlings, 1990). The overall intensity of predation on egg capsules at different locations, therefore, may reveal little about the selective force(s) responsible for the production of thick- and thin-walled capsules. Instead, site-differences in the presence and abundance of specific types of capsule-eating predators (i.e. predators that differentially open thin-walled capsules) may have the biggest impact on capsule morphology.

Although the thickness of the capsule wall clearly influences its effectiveness as a defense, the microstructural composition of the capsule walls must also play a substantial role. Egg capsules of higher caenogastropods are structurally complex (Tamarin and Carriker, 1967; Bayne, 1968; Sullivan and Maugel, 1984; Hawkins and Hutchinson, 1988; D'Asaro, 1988), and capsules of taxonomically-related gastropods share similar microstructural characteristics (D'Asaro, 1988). Muricid egg capsules, such as those of *Nucella emarginata*, are composed of three to four laminae (Tamarin and Carriker, 1967; Bayne, 1968; Sullivan and Maugel, 1984; D'Asaro, 1988; Rawlings, 1990), yet little is known about the functional properties of these structures. Some laminae are more permeable to small solute molecules than others (Pechenik, 1982; 1983). Other capsule laminae also appear more effective at preventing micro-organisms, such as bacteria and protists, from entering the capsule chamber. In the present study, for instance, embryos within capsules stripped of their fibrous outer laminae (L1 and L2) were attacked by protists, organisms unable to enter the chamber of whole capsules (Lord, 1986; Rawlings, 1995). This differential vulnerability undoubtedly reflects differences in both the thickness and structural composition of the outer and inner laminae. By understanding more about the functional role of each lamina within the capsule wall, therefore, it may be possible to assess the significance of intra- and interspecific variation in capsule wall microstructure.

Subtle differences in capsule morphology can clearly have substantial effects on the survival of encapsulated embryos. However, other aspects still require investigation. Intraspecific differences in the wall thickness of intertidal egg capsules have the potential not only to influence the susceptibility of encapsulated embryos to intertidal predators, but also to influence their susceptibility to such environment risks as: desiccation (Pechenik, 1978; Rawlings, 1992; Chapter 4), salinity stress (Pechenik, 1982; 1983), and ultraviolet radiation (Chapter 5). Thick-walled capsules may also entail significant costs, including the energetic costs of production (Perron, 1981) and diffusional constraints associated with oxygen and carbon dioxide transfer (Strathmann and Chaffee, 1984; Chapter 7). If the adaptive significance of variation in capsule form within the Gastropoda is to be understood, therefore, the benefits and costs associated with these differences must be assessed.

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Table 3-1. Cumulative total of the first capsules to be opened per cage by *G. oregonense* individuals when given a choice between thin-walled capsules from Ross Islet and thick-walled capsules from either Kirby Point or Grappler Inlet.

	Experiment I				Experiment IIA				Experiment IIB			
Isopods	Ross (Thin)	Kirby (Thick)	N	P*	Ross (Thin)	Kirby (Thick)	N	P	Ross (Thin)	Grappler (Thick)	N	P
Female:	-	-	-		12	1	20		11	1	20	
Male:	-	-	-		4	1	16		3	1	16	
Total:	15	2	31†	<0.01	16	2	36	<0.01	14	2	36‡	<0.01

* Results of a χ^2 analysis comparing the number of thick versus thin-walled capsules eaten first based on combined totals for male and female isopods

† Four of 35 isopods died over the 30-d experiment

‡ Eight of the 80 isopods died over the 50-d experiment

Table 3-2. Cumulative total of the first capsules to be ruptured and subsequently emptied by *G. oregonense* individuals, when given a choice between stripped capsules from Ross Islet (originally thin-walled) and Kirby Point (originally thick-walled). Thirty isopods were each allocated one stripped capsule from Ross Islet and Kirby Point and daily observations were recorded on the first capsule to be ruptured and the first capsule to be emptied of contents within each cage. Ties refer to cases where both capsules per cage were ruptured or emptied on the same day. Sample sizes (N) indicate the total number of isopods that survived to rupture or empty at least one capsule per cage.

	First Selected Capsule:				
Capsule fate	Ross (Stripped)	Kirby (Stripped)	Ties	N	P†
Ruptured:	15	7	8	30	>0.1
Emptied:	12	13	2	27‡	>0.95

†Results of a χ^2 analysis comparing the total number of Ross versus Kirby capsules opened per cage. Ties were treated statistically by assuming that each capsule morph was ruptured (or emptied) first in 50% of the ties.

‡Three isopods died before either capsule within a cage was emptied.

Figure 3-1. Cage types used to examine predation on *Nucella emarginata* egg capsules by the isopod *Gnorimosphaeroma oregonense*: a) Microfuge cage, modified microcentrifuge vial (10 x 10 x 15 mm); b) Minifuge cage, modified plastic cylinder (20 x 20 x 25 mm), showing the position of a whole and stripped capsule in the Tygon™ holdfast. Both cages had mesh-paneled bases.

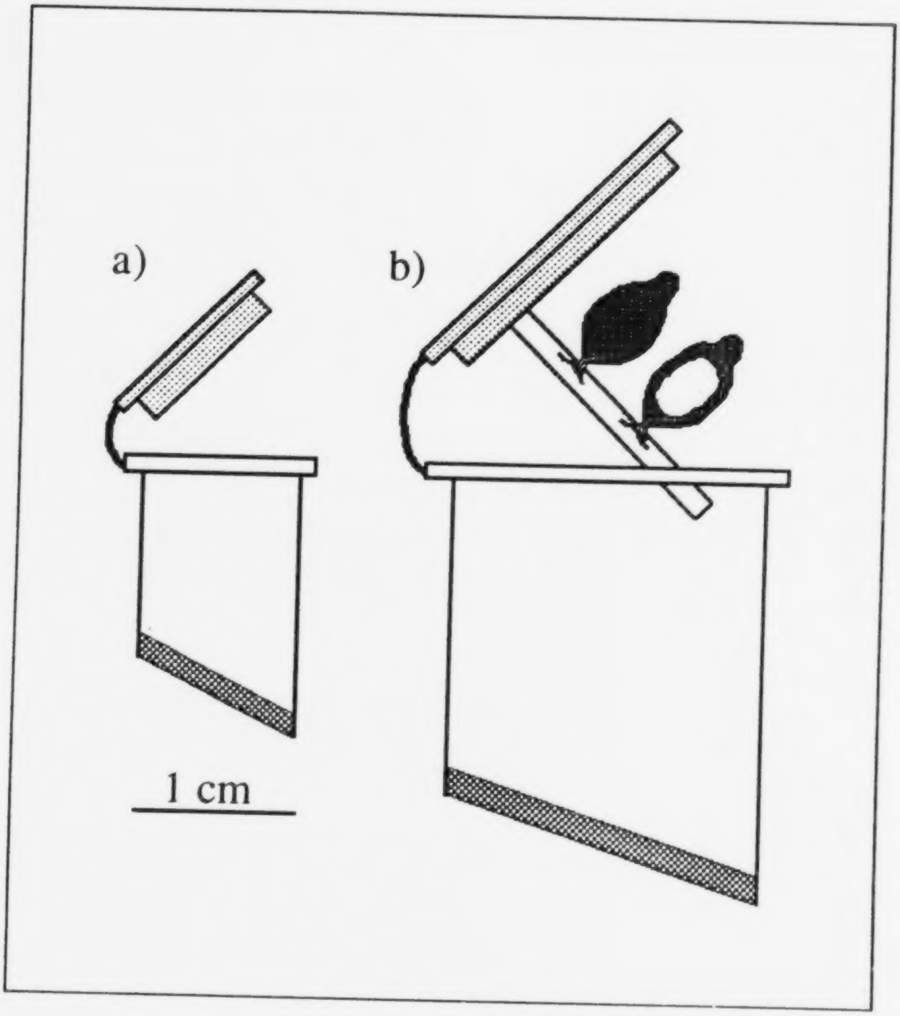


Figure 3-2. Comparison of mean capsule wall thickness among eight populations of *Nucella emarginata* separated along a wave-exposure gradient in Barkley Sound, British Columbia. Symbols represent the mean (± S.E.) of ten capsules collected from each site. The results of a Tukey Multiple Comparison Test comparing mean capsule wall thickness among populations are shown in the inset; sites connected by a line are not significantly different from one another at $\alpha = 0.05$. Abbreviations for collection sites refer to: GR: Grappler Inlet (48°49'54" N, 125°06'54" W); RI: Ross Islet (48°52'12" N, 125°09'42" W); SP: Self Point (48°50'54" N; 125°09"42" W); KB: Kelp Bay (48°51'48" N; 125°06'18" W); WZ: Wizard Rock (48°51'24" N; 125°09'36" W); VP: Voss Point (48°49'48" N; 125° 11'18" W); KP: Kirby Point (48°50'42" N; 125°12'24" W); FG: Folger Island (48°49'48" N; 125°15'00" W).

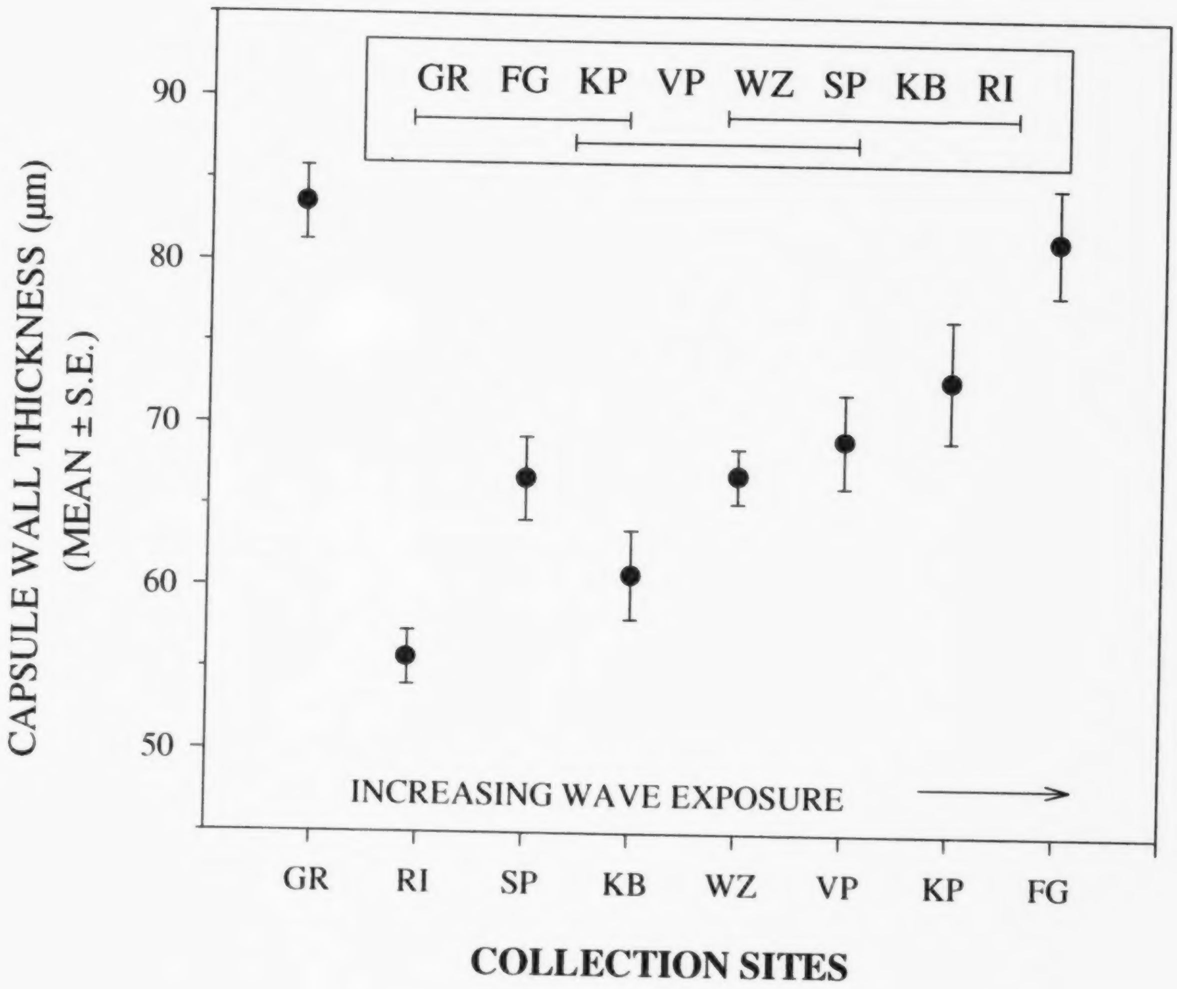

GR FG KP VP WZ SP KB RI
CAPSULE WALL THICKNESS (µm)
(MEAN ± S.E.)
90
80
70
60
50
INCREASING WAVE EXPOSURE
GR
RI
SP
KB
WZ
VP
KP
FG
COLLECTION SITES

Figure 3-3. Cumulative total of the first *Nucella emarginata* capsules to be opened per cage by *Gnorimosphaeroma oregonense* individuals over 30 d (a: Experiment I) and a 50 day period (b: Experiment II), when given a choice between a thin-walled or thick-walled egg capsule (a: Ross vs. Kirby; b: Ross vs. Kirby, and Ross vs Grappler). Experimental cages, containing a single isopod and two capsules, were checked every 2-4 d. Capsules were opened in 17 of 31 cages and 34 of 72 cages in Experiment I and II, respectively.

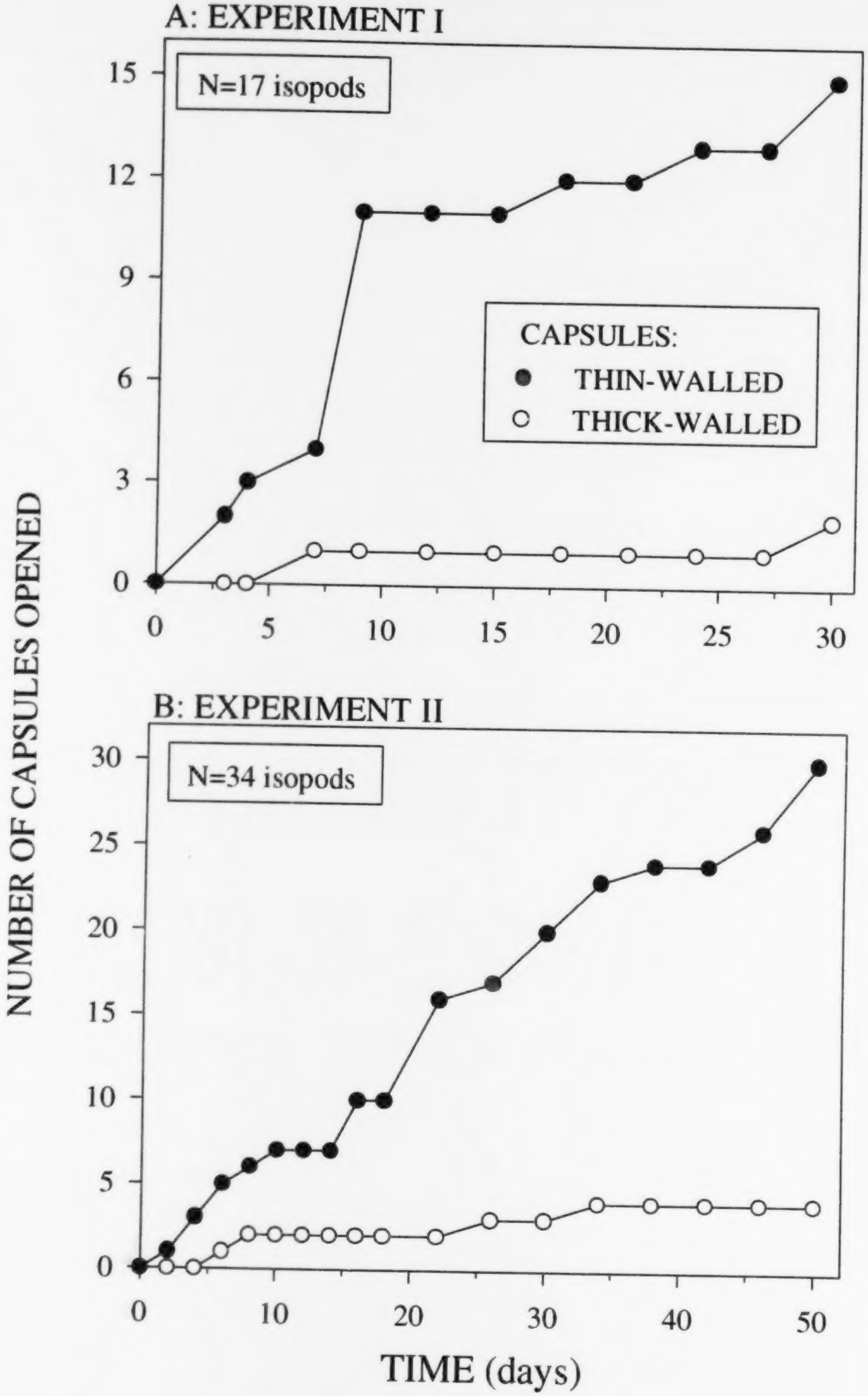

A: EXPERIMENT I
N=17 isopods
15
12
9
6
3
0
0
5
10
15
20
25
30
CAPSULES:
● THIN-WALLED
○ THICK-WALLED
B: EXPERIMENT II
N=34 isopods
30
25
20
15
10
5
0
0
10
20
30
40
50
NUMBER OF CAPSULES OPENED
TIME (days)

Figure 3-4. Number of days required by *Gnorimosphaeroma oregonense* individuals to open thick- versus thin-walled egg capsules of *Nucella emarginata*. Eighty isopods were given two capsule morphs simultaneously and the number of days required to open each capsule was recorded. Capsules were only opened by 34 isopods. Each symbol indicates the results from one isopod. The diagonal line represents an equal rate of predation on thick and thin-walled capsules; a symbol falling above the line indicates that within a cage an isopod took longer to open a thick-walled capsule; a symbol falling below the line indicates that an isopod took longer to open a thin-walled capsule. Open symbols represent cages where one capsule was opened but the other hatched before being ruptured; hence, the time taken to open the hatched capsule is underestimated. For open symbols above the line, embryos within thick-walled capsules hatched before being opened, whereas for open symbols below the line, embryos within thin-walled capsules hatched before being opened.

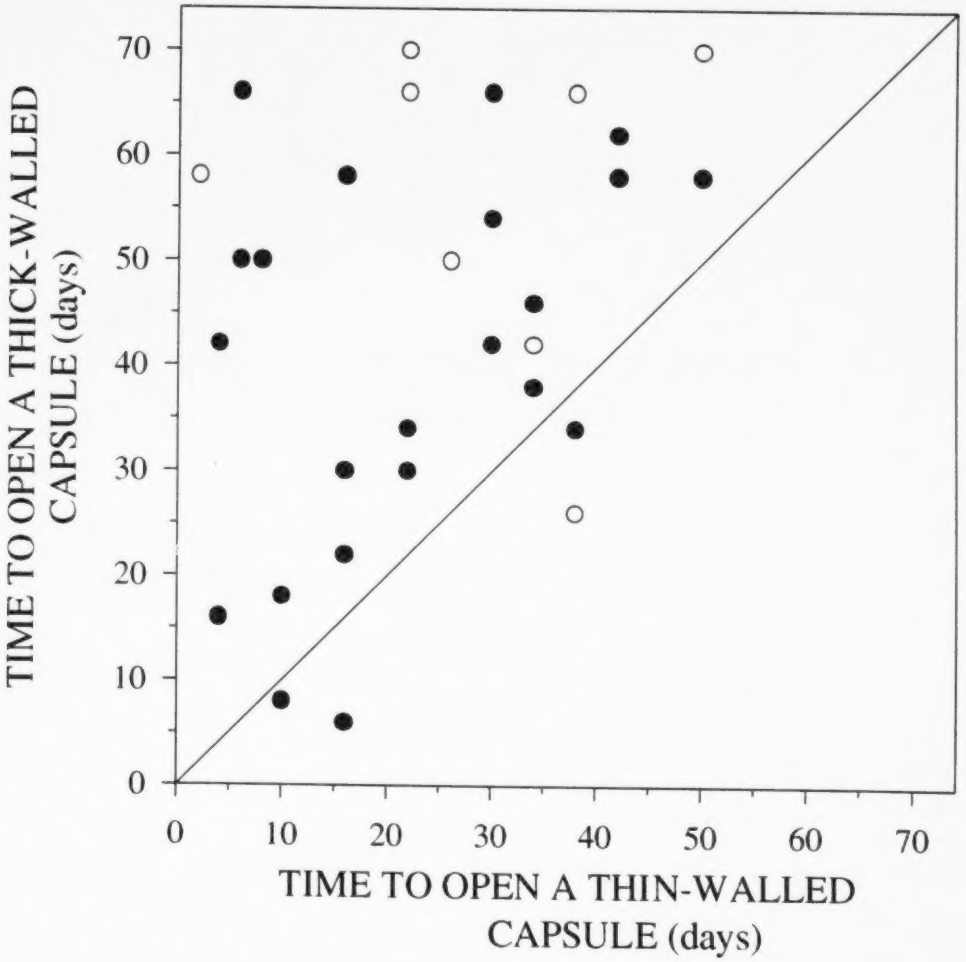


Figure 3-5. Rate of consumption of pieces of capsule wall from thick- and thin-walled capsules of *Nucella emarginata* by *Gnorimosphaeroma oregonense* individuals. Symbols represent the mean (± S.E.) amount of material eaten after each time interval for 19 isopods. As one isopod ate all of one piece of thin-walled capsule on day 21, and this piece was not replaced, these data were omitted from this figure. Hollow symbols (controls) refer to the weight change of capsule pieces not exposed to isopod predators (n=10); negative values indicate that control pieces gained weight.

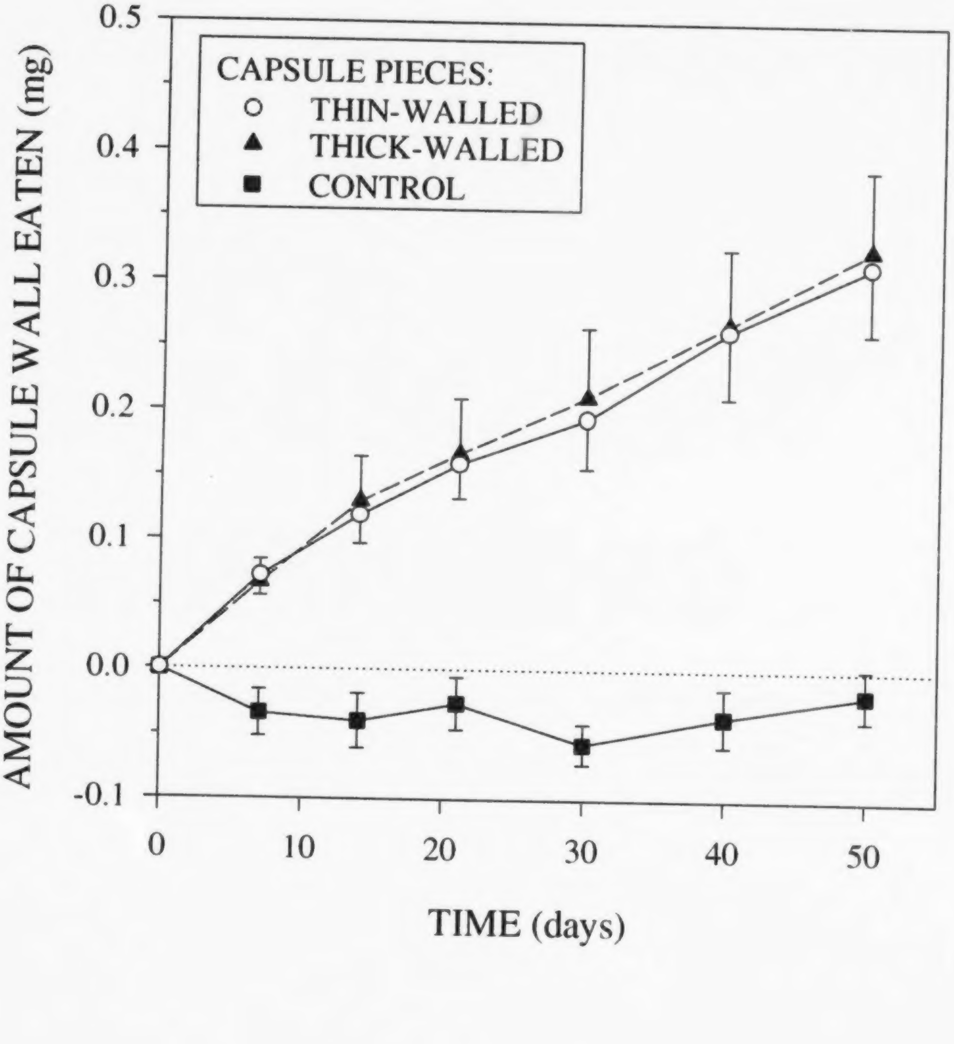
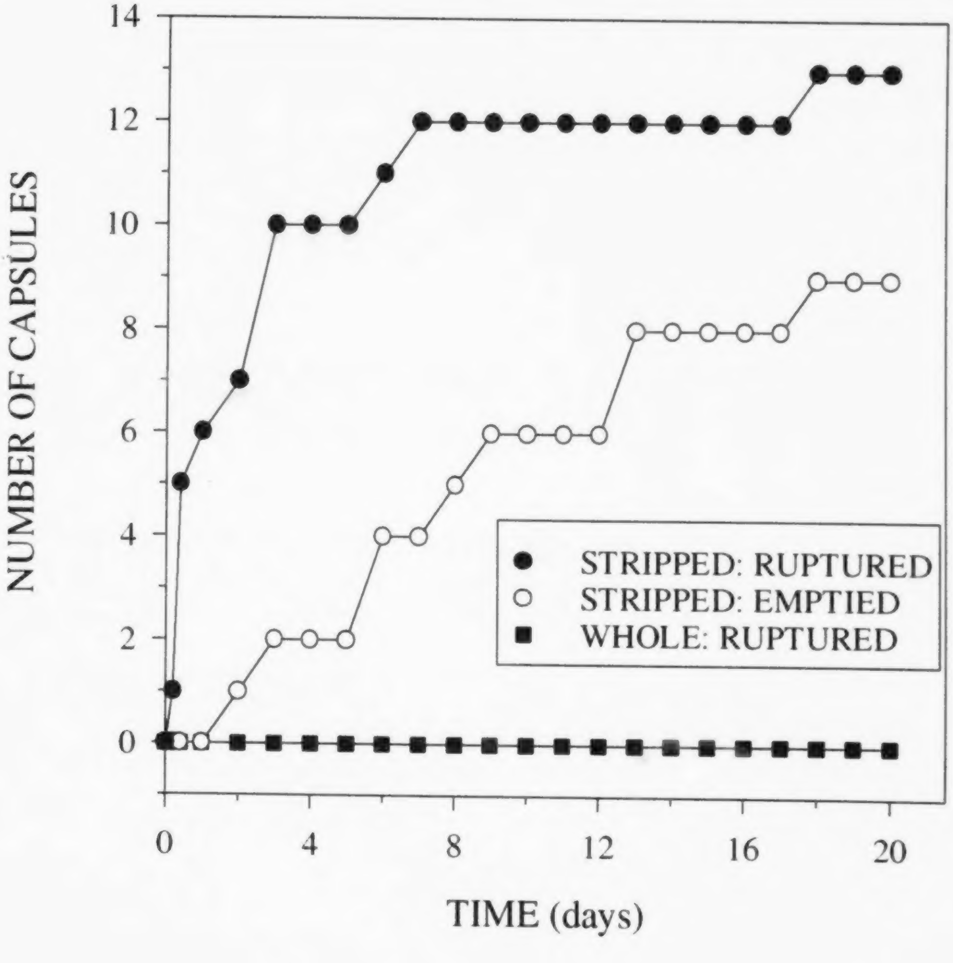


Figure 3-6. Cumulative predation on stripped and whole *Nucella emarginata* egg capsules by *Gnorimosphaeroma oregonense* females. Each isopod (n = 15) was given one stripped and one whole egg capsule from Kirby Point, and capsules were checked daily for 20 d. Capsules opened by isopods were classified as either ruptured (fluid leaking from the chamber) or emptied (nurse eggs and embryos absent from the chamber).



CHAPTER 4

Through thick and thin: Resistance of the egg capsules of the rocky shore marine gastropod *Nucella emarginata* to desiccation

Abstract

Although many adult intertidal organisms exhibit adaptive structural, behavioral, and physiological modifications in response to desiccation stress, relatively little is known about the ability of embryonic and juvenile stages to avoid, resist, or tolerate water loss. This is surprising since interspecific differences in the ability of these developmental stages to withstand desiccation stress can have important consequences for the survival and distribution of organisms within the intertidal zone. The deposition of eggs within tough, multilaminated capsules or gelatinous masses may be one means of protecting embryos from such physical stresses as desiccation during their development within benthic intertidal habitats. To investigate this, I examined 1) the resistance of egg capsules of the rocky shore marine gastropod, *Nucella emarginata*, to desiccation under both laboratory and field conditions, and 2) the ability of encapsulated embryos of this species to tolerate extensive water loss from the capsule chamber. In addition, because capsules collected from different populations of this species exhibit significant variation in the size, shape, and thickness of their walls, I used a simple desiccation model to simulate the impact of this variation in capsule form on rates of water loss, and the survival of developing embryos.

Like egg capsules of other neogastropod species, the walls of *Nucella emarginata* capsules were not impervious to water loss. Capsule walls did have a 10-fold lower diffusivity to water vapor than air, indicating that these structures do provide embryos with considerable protection from desiccation, however, the resistance of capsule walls to water loss was substantially lower than published values for the egg coverings of terrestrial organisms. Also, because the capsule wall resistance to water loss was lower than the boundary layer resistance over wind velocities from 0 to 1.5 m/s, rates of water loss depended greatly on the movement of air around the capsule. The effect of wall thickness on water loss from these capsules also varied depending on the range of wind velocities to which capsules were exposed. At low wind velocities, thick capsule walls had little effect on reducing the rate of desiccation rate relative to thin capsule walls. At higher wind velocities, however, the thickness of the capsule wall became a rate-limiting factor due to the decreasing thickness of the boundary layer surrounding these capsules.

Nevertheless, given the low wind speeds experienced by capsules under natural field conditions, and the overriding influence of capsule surface area / volume ratios on the time taken for capsules to dehydrate, intraspecific differences in capsule wall thickness appear unlikely to represent adaptive responses to desiccation stress.

In contrast, encapsulated embryos of *N. emarginata* were remarkably tolerant to the loss of water from the capsule chamber. Late-stage embryos survived up to 80% water loss from the capsule chamber and thus appeared well adapted to withstanding the tremendous flux in salinity associated with desiccation. Embryonic survival during the frequent and prolonged exposure of *N. emarginata* egg capsules to air may therefore depend primarily on the deposition of these structures within humid, wind-sheltered microhabitats, and on the remarkable tolerance of embryos to dehydration, rather than on specific properties of the capsule wall itself.

Introduction

To survive within the intertidal zone of marine environments, plants and animals must be able to withstand physical stresses associated with their periodic exposure to air. The ability of organisms to resist or tolerate desiccation stress can have a profound effect on their survival (Schonbeck and Norton, 1978; Menge, 1978a), vertical distribution (e.g., Kensler, 1967; Foster, 1971; Schonbeck and Norton, 1978; Vermeij, 1978), and growth / productivity within the intertidal zone (e.g., Menge, 1978b; Beer and Eshel, 1983; Oates, 1985, 1986; Maberly and Madsen, 1990; Madsen and Maberly, 1990). The relative resistance of organisms to water loss may also influence patterns of succession following disturbance events (see Schonbeck and Norton, 1978; Buschmann, 1990) and competitive interactions between species (e.g., Wethey, 1984). Differences in the intensity of desiccation stress among locations can thus have an important effect on the distribution and abundance of intertidal organisms.

Adaptive structural, behavioral, and physiological modifications in response to the rigors of desiccation stress are commonplace among intertidal organisms (see Vermeij, 1978). Some species, for instance, actively avoid desiccation stress by remaining within moist crevices or microhabitats during emersion (e.g., Kensler, 1967; Wolcott, 1973; Menge, 1978a). Others have developed elaborate mechanisms for sealing themselves off from the environment using mucus barriers (e.g., limpets; Wolcott, 1973) or through the controlled use of their opercular valves (e.g., barnacles; Foster, 1971). Changes in body shape (reduced surface area / volume) and shell form also appear to be common adaptive responses among higher intertidal gastropods (Vermeij, 1973; 1978). Likewise, many high intertidal organisms have developed an amazing ability to survive severe dehydration (Allanson, 1958; Kensler, 1967; Wolcott, 1973), including several limpet species that can withstand losses exceeding 80% of their total body water (including extravisceral water; Wolcott, 1973).

Some intertidal algae also exhibit a remarkable capacity to survive extended periods of emersion within high intertidal environments. This can result from reduced rates of water loss associated with variation in cell wall thickness (parenchyma cells: Zaneveld, 1937; Kristensen, 1968, but see Dromgoole, 1980), biochemical composition (e.g., hygroscopic polysaccharides: Bérard-Therriault and Cardinal, 1973), and shape (e.g., Dromgoole, 1980; Oates, 1985; 1986). Such inter- and intraspecific differences in resistance to water loss, and/or recovery following desiccation stress, may also directly reflect the probability of exposure to desiccation stress (Zaneveld, 1937; Jenik and Lawson, 1967; Schonbeck and Norton, 1978; Dring and Brown, 1982; Oates and Murray, 1983). For many intertidal algae, however, high surface areas required for photosynthesis are usually not compatible with the ability to avoid tissue water loss, except in those species with substantial reservoirs of water (Dromgoole, 1980; Oates, 1985; 1986). Instead, survival within the high intertidal zone results from increased tolerance of desiccation (Schonbeck and Norton, 1979). For instance, some algae can withstand > 75% water loss from their tissues without suffering irreversible damage (Schonbeck and Norton, 1979; Dring and Brown, 1982; Madsen and Maberly, 1990), and can quickly recover their ability to photosynthesize once reimmersed (Dring and Brown, 1982; Madsen and Maberly, 1990). Proportional losses of water such as this are not unrealistic over periods of emersion on warm sunny days along temperate shorelines (e.g., Kanwisher, 1957; Kristensen, 1968; Kraemer, 1990).

Although considerable attention has been devoted towards examining the ability of adult intertidal plants and animals to withstand desiccation stress associated with periodic emersion, few studies have examined the vulnerability of embryos and juveniles to such physical stresses (but see Foster, 1971; Brawley and Johnson, 1991). Planktonic larvae of benthic invertebrates and algae must successfully settle, metamorphose / germinate, and grow within this habitat for populations to become established within the intertidal zone. Somewhat surprisingly, however, little is known about the morphological, behavioral, or physiological adaptations exhibited by larval and juvenile stages to desiccation (see Underwood and Denley, 1984), even though interspecific differences in the tolerances of these developmental stages can have an enormous effect on the distribution and abundance of intertidal organisms. Since many juveniles die within the first few days following settlement in the intertidal zone, their resistance to physical stresses may be very limited (Foster, 1971; Connell, 1985; Brawley and Johnson, 1991; Gosselin and Qian, in review). Survival from desiccation stress may thus depend on the availability of specific microhabitats where water loss can be minimized (Brawley and Johnson, 1991). Clearly, a better understanding of the vulnerability of larvae and juveniles to desiccation stress is necessary if the role of desiccation in governing the abundance and distribution of intertidal organisms is to be assessed.

Tough, multilaminated capsules or gelatinous masses may be one means of protecting embryos from physical stresses during their development within benthic marine habitats. These structures are common among such phylogenetically diverse marine organisms as flatworms, polychaetes, molluscs and chordates, and the variety of egg coverings produced both within and among these phyla is startling. There are many potential benefits associated with the deposition of eggs within these structures. Egg capsules of marine gastropods, for instance, can protect developing embryos to some degree from specific predators (e.g., Spight, 1977; Brenchley, 1982; Rawlings, 1990; 1994), osmotic stress (Pechenik, 1982; 1983), ultraviolet radiation (Chapter 5), and attack by bacteria and protists (Lord, 1986; Rawlings, 1995). For those species depositing egg capsules within the intertidal zone, therefore, capsule walls and egg mass jelly may also protect developing embryos from water loss.

Evidence that egg gastropod capsules and jelly masses protect embryos against desiccation stress remains equivocal. Egg capsules and masses of many gastropod species appear to desiccate rapidly in air (Bayne, 1968; Pechenik, 1978). Likewise, desiccation has frequently been implicated as a primary cause of embryonic mortality within field-deposited egg capsules (e.g., Emlen, 1966; Feare, 1970; Spight, 1977), although direct evidence for this has often been lacking. In contrast, anecdotal observations suggest that egg coverings of some species may substantially protect embryos from desiccation (Holle and Dineen, 1957, Fretter and Graham, 1962). These conflicting results may thus be a consequence of the tremendous diversity of egg coverings produced by marine gastropods. The form of these egg coverings can vary markedly among families (e.g., D'Asaro, 1988, 1991), among closely-related species (e.g., Bandel, 1973; Palmer et al., 1990; Collins et al., in review), and even among populations of the same species (Rawlings, 1990; 1994; Chapter 2). Hence, variation in the properties and morphologies of capsular cases and egg mass jelly may be associated with differences in the ability of these structures to protect developing embryos from desiccation stress.

In the present study I examined the resistance of benthic egg capsules of the marine gastropod, *Nucella emarginata* (northern species, Palmer et al., 1990), to desiccation.

These snails are common inhabitants of rocky intertidal shores from Alaska to California. Eggs are deposited year-round within 6-10 mm-long vase-shaped egg capsules, which are attached to firm substrata in the intertidal zone. Each capsule can contain up to 55 embryos (Rawlings, unpub. data) which spend 2-3 months developing within the capsule before emerging as juvenile snails (see Strathmann, 1987). Capsule size, shape, and wall thickness also vary significantly among populations of this species (Rawlings, 1990; 1994). Because the capsule wall represents the primary barrier separating developing embryos from their surroundings, differences in wall thickness may substantially affect the rate of water loss from the capsule chamber. Likewise, changes in capsule size and shape (surface area / volume) can also affect the time taken for capsules to dehydrate. The objectives of this study were: 1) to compare the rate of water loss from capsules collected from three intertidal populations of *N. emarginata* under laboratory conditions of still and moving air, 2) to determine the relative resistances of the capsule wall and boundary layer to water loss, 3) to examine the ability of encapsulated embryos of *Nucella emarginata* to tolerate dehydration, and 4) to determine the relative importance of capsule wall thickness, capsule size, and capsule shape, on rates of water loss from *N. emarginata* capsules using a simple desiccation model.

Materials and Methods

Rates of water loss from *Nucella emarginata* egg capsules

I examined the resistance of *Nucella emarginata* egg capsules to desiccation by measuring rates of water loss in the laboratory under conditions of still and moving air. Capsules used in these experiments were collected from three intertidal populations: Grappler Inlet (48°49'54" N; 125°06'54" W), Ross Islet (48°52'12" N; 125°09'52" W), and Kirby Point (48°50'42" N; 125°12'24" W). These specific sites were selected based on capsule morphology (Table 4-1), as well as the local availability of egg capsules at the time these experiments were conducted. To ensure that capsules were clean and undamaged, only freshly-deposited capsules were removed from each site. Once collected, these capsules were kept in site- and clutch-specific mesh-paneled bags, and suspended in running seawater until needed. Only one capsule per clutch was tested within a given set of experiments to ensure independence among replicate capsules. Prior to testing, each capsule was placed within a Petri dish of sea water and allowed to equilibrate to ambient temperature. This ensured that artificially inflated desiccation rates did not occur due to initial temperature differences between the capsule and environment.

a) Desiccation rates in still air

Preliminary measurements: To examine the time interval over which capsules lost water and the rate of water loss over time, I measured the desiccation rate of individual capsules under ambient laboratory temperatures and humidities. For each trial, I mounted an individual capsule in a small holder to keep it upright, and placed the capsule and holder in the enclosed weighing chamber of a Mettler analytical balance. The temperature and relative humidity (rh) within the weighing chamber were recorded with a thermohygrometer (Oaklon Thermohygrometer Model 37200-00) prior to placing each capsule inside, and immediately following the removal of the capsule. Capsule weight was recorded at 5 - 10 minute intervals until weight change was less than 0.05 mg / 10 minute period. These capsules were then placed in a desiccator (37% rh @ 20°C), in which conditions were similar to the weighing chamber, and dried to a constant weight. A constant humidity was maintained by incubating a known density of sulfuric acid within a sealed desiccator at 20°C; these conditions were confirmed by mounting a thermohygrometer within the lid of the desiccator. To determine the amount of volatile water within the capsule, the final dry weight of the capsule was subtracted from the initial starting wet weight The rate of water loss was examined by plotting the amount of volatile water lost over time for these capsules.

Among-population comparisons: To examine the desiccation characteristics of capsules collected from Grappler Inlet, Ross Islet, and Kirby Point, I compared rates of water loss under conditions of still air and controlled humidity (37%) and temperature (20 °C). These conditions were generated within a desiccator, as described above, and were chosen because they established a high concentration gradient in water vapor density across the capsule wall, and also were similar to ambient laboratory conditions under which experiments in moving air were conducted (see below). Other studies have shown that these conditions may be realistic even within temperate intertidal habitats (Schonbeck and Norton, 1980). Three capsules, one from each study population, were dried simultaneously within the desiccator. Prior to drying these capsules, I measured the body length and chamber width of each capsule using a dissecting microscope equipped with an ocular micrometer. These measurements allowed the surface area (SA) and volume (VOL) of the capsule body to be estimated using the formula for a prolate ellipsoid: $SA = 2\pi(b/2)^2 + (2\pi(a/2)(b/2))(\sin^{-1}\varepsilon)/\varepsilon$, and $VOL = 4/3\pi (a/2)(b/2)^2$; where a and b are the average capsule body length and chamber width, respectively, and ε, the eccentricity, $= [((a/2)^2 - (b/2)^2)^{0.5}]/a$. Each capsule was then damp dried with an absorbent tissue, weighed, and mounted immediately onto a capsule holder within the flask; this holder served to keep capsules upright and spaced well apart to minimize any interference between localized humidity gradients generated around each capsule. Desiccation trials were conducted for a period of 50 minutes, during which time capsules lost from 30 - 53% of their volatile water; results from preliminary trials (see above) indicated that desiccation rates were relatively constant over this range of water loss. Capsules were then reweighed, placed in small desiccation flasks (37% rh @ 20°C), and dried to a constant weight. A total of 15 capsules from each site were tested using this experimental procedure.

b) Desiccation rates in moving air

The desiccation rate of capsules was examined under conditions of moving air by placing them within a wind tunnel. This wind tunnel was based on the "closed circuit, open jet" design of Vogel (1969) and was constructed out of 3" and 4" plastic pipes, and powered by a 12 volt DC motor driving two 3" fans (Fig. 4-1). This system gave a fairly wide working section, with stable wind velocities across the width. Capsules were mounted within the wind tunnel using a Plexiglas stand positioned across the working section. To ensure that capsules remained upright while drying, the stalk of each capsule was inserted into a small hole drilled in the surface of the Plexiglas. Any capsules that fell over during an experiment were discarded. Only one capsule was desiccated within the wind tunnel at once, although capsules from each study population were interspersed to ensure that capsules from all sites were exposed to the same range of environmental conditions. A hot-wire anemometer (Kurtz Mini Anemometer: 490 series) was used to record wind speed. This was positioned 20 mm away and slightly downstream from the mounted capsule to ensure minimal interaction between the desiccating capsule and probe. A themohygrometer, mounted in the downstream half of the working section, was also used to record air temperature and humidity at the beginning and end of each experiment. All experiments were conducted under ambient laboratory conditions of humidity and temperature.

Preliminary measurements: Preliminary trials were also necessary to estimate the approximate length of time over which to conduct these experiments. Three capsules from each population were tested in the wind tunnel using set wind speeds of ≈ 0.5, 1.0, and 1.5 m/s. These wind speeds spanned the range measured by Wolcott (1973) at a height of 10 mm off the substratum in the intertidal zone at the Bodega Marine Laboratory. As before, the dimensions and wet weight of each capsule were measured prior to placing capsules within the wind tunnel. To determine the rate of desiccation over time, each capsule was removed from the wind tunnel at five minute intervals and reweighed. The analytical balance was located immediately adjacent to the wind tunnel to minimize the time required to remove, weigh, and reposition the capsule within the air stream. Measures of wind speed, temperature and humidity were recorded every five minutes and averaged over the duration of the trial. Each trial was continued until the amount of water lost per capsule was less than 0.05 mg per five minute period. Capsules were then placed in a desiccator (37% rh @ 20°C), dried to a constant weight, and reweighed.

Among-population comparisons: The desiccation rate of capsules was compared among different populations over a range of wind speeds from 0 - 1.5 m/s. Each capsule was measured and weighed prior to placing it within the wind tunnel for 15 minutes. Wind velocity readings were recorded at one minute intervals and averaged for each trial. Because temperature and humidity varied little over each trial, readings were taken only prior to and immediately following each experiment. Twenty capsules were tested for each population.

Mortality of encapsulated embryos exposed to desiccation stress

To relate the loss of water from *N. emarginata* capsules to embryonic survival, I examined the tolerances of embryos to desiccation stress. Capsules were collected from Wizard Rock (48°51'24" N; 125°09'36" W) in February, 1992. Once in the laboratory, I categorized capsules into two groups according to the developmental stage of their encapsulated embryos. Embryos that were at the third veliger stage or earlier (see LeBoeuf, 1971) were defined as "early-stage embryos"; capsules containing these developmental stages could be identified by the presence of large numbers of nurse eggs that were partially visible through the capsule wall. Embryos start to consume nurse eggs once they reach the third veliger stage (LeBoeuf, 1971; Rawlings, pers. obs.). Embryos at the fourth-veliger stage were defined as "late-stage embryos". These were usually large enough to be visible through the capsule wall, and few if any nurse eggs were present at this developmental stage.

Although the tolerances of early- and late-stage embryos were conducted in two separate experiments, the procedures followed for both were identical. Prior to subjecting each capsule to desiccation stress, capsules were damp dried and weighed using a Mettler analytical balance ("wet wt."). Capsules were then exposed to desiccation stress for varying intervals of time. This was achieved by placing 4 - 5 capsules within a desiccator (@ 80% rh, 18°C) for period of 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, and 10 h. These environmental conditions ensured that capsules dried relatively slowly, and hence, allowed capsules at different stages of dehydration to be examined. Capsules were spaced well apart within the incubator using a capsule holder, as described previously. Once capsules had dehydrated for their set period of time, they were reweighed ("wt. after desiccation"), placed in mesh bags, and reimmersed in flowing seawater. In total, 67 capsules containing early-staged embryos, and 70 capsules containing late-staged embryos were tested in this experiment.

Embryos were allowed to recover from this experiment for varying periods of time depending on their developmental stage before assessing survival. Late-stage embryos were examined three days following their exposure to desiccation stress. At this time, capsules were opened, live and dead embryos counted, and then capsular cases, embryos, and nurse eggs, dried to constant weight in a desiccator flask at 80% rh ("capsule dry weight"). An embryo was considered dead or dying if : 1) tissue was necrotic, 2) the visceral mass exhibited an extensive purple color, 3) cilia were not beating in the velar lobes, 4) yolk was leaking from the visceral mass, and 5) the larval shell was extensively damaged. Usually two or more of these characteristics were evident in dead embryos. Since velar cilia may beat even in detached portions of *Nucella* embryos (Rawlings, pers. obs.), ciliary activity alone was not used to confirm embryonic survival. Early-stage embryos were kept in mesh bags for 15 days following desiccation stress. This protracted period of time was necessary simply because embryonic mortality in these young embryos was more difficult to assess. After 15 days, dead embryos were readily apparent. In some cases, the only remains of these embryos were their larval shells.

The percentage of volatile water lost from the capsule chamber was determined using the following equation:

$$\text{\% volatile water lost} = \frac{(\text{wet wt - wt after desiccation})}{(\text{wet wt - capsule dry wt})} \times 100 \qquad (1)$$

This method may have overestimated the amount of water lost from the capsule during desiccation by underestimating the capsule volume. For instance, over the 3 - 15 days before embryonic mortality was quantified, healthy embryos may have added substantially more calcium to their larval shells (i.e., inflated the capsule dry weight). Nevertheless, given the proportional contribution of the dry weight of the capsular case and embryos to the capsule wet weight (on average: 20.2%, n = 50), the error associated with small changes in embryo dry weight alone was not likely to be substantial.

Control experiments were also conducted to determine a) the mortality of embryos within capsules prior to their exposure to desiccation stress ("collection control"), and b) the mortality of embryos resulting from extended incubation at 18°C ("temperature control"). For the collection control, 19 capsules containing early-stage embryos and 20 capsules containing late-stage embryos, were collected from the field, along with the experimental capsules (see above). These capsules were opened immediately upon return to the laboratory, and the percentage of embryos alive within each capsule was determined. For the temperature control, two additional sets of 10 capsules containing early-stage embryos and late-stage embryos were weighed, and then placed within a desiccator (@ 100% rh, 18°C) for 10 h. Following this, capsules were reweighed, and assigned to mesh bags for 3 or 15 days, depending on their developmental stage (see above). Capsules were then opened and embryonic survivorship assessed. Capsule cases and contents were dried to constant weight, and the percentage of volatile water lost from the capsule chamber was assessed. These capsules lost, on average (± SE), 4.0 ± 0.75% of their internal volume over the 10 h incubation period at 100% rh.

Models of water loss from egg capsules

To determine the effect of capsule wall thickness on rates of water loss and embryonic survival, I modeled water loss from cylindrically-shaped egg capsules using parameters derived from real capsules of *N. emarginata*. Because this model assumes that water is lost only through the sides of the cylinder, and not through the two ends, this should approximate water loss from real capsules more closely than a spherical model, since *N. emarginata* capsules are capped on one end by a thick mucus plug and on the other end by a tough stalk. The equation used to describe the rate of mass flux from these capsules is :

$$M = (A\ \Delta C) / (r_b + r_w) \tag{2}$$

(see Gates, 1980), where M is the rate of mass loss per unit time in g/s, A is the surface area in m^2; ΔC is the concentration gradient of water vapor density across the capsule wall in g/m^3, r_b is the boundary layer resistance, and r_w is the capsule wall resistance to water vapor, in s/m. This equation can be rewritten as:

$$M = (A\ \Delta C) / [(1/h_m)+(T/D_w)] \tag{3}$$

where h_m is an empirically derived term, the mass transfer coefficient in m/s, which describes the ease of movement of water vapor across the boundary layer surrounding an object, T is the thickness of the capsule wall in m, and D_w is the diffusion coefficient of the capsule wall to water molecules in m^2/s. This equation can be rewritten for water loss from an open-ended cylinder as:

$$M = \Delta C / [(1/(2 \pi h_m L R_o)) + (\ln(R_o/R_i))/(2 \pi D_w L)] \qquad (4)$$

(White, 1988), where R_o is the outer capsule radius, R_i is the inner capsule radius, and L is the length of the cylinder.

Hence, to model the effect of changing the wall thickness, $\ln(R_o/R_i)$, on the rate of water loss from capsules, it was first necessary to measure the diffusion coefficient for capsule wall material (D_w) and the mass transfer coefficient (h_m).

a) Estimates of the diffusion coefficient of capsule wall material

Diffusion coefficients describe the passage of a molecule through a specific medium by purely diffusive processes, and depend on both the molecular species and medium though which it is passing. I estimated the diffusion coefficient of *N. emarginata* capsule walls using "diffusion chambers" (Pechenik, 1982) made from polystyrene disposable spectrophotometer cuvettes and pieces of Plexiglas. Holes (≈ 1 mm diam.) were drilled 10 mm above the bottom of each cuvette, in the midline. Holes of equal size were also drilled in matching pieces of Plexiglas (1 mm thick), such that when each cuvette and Plexiglas piece were superimposed, these holes overlapped perfectly. A piece of capsule wall, cut from a fresh capsule and rinsed in distilled water, was sandwiched between these two holes by clamping the cuvette and Plexiglas cover together. Each diffusion chamber was filled with 4 ml of fresh water and sealed with a tight covering of Parafilm. Diffusion chambers were then weighed, placed in a desiccator (37% rh @ 18°C), and left for a period of 15 - 16h. Following this incubation period, the diffusion chambers were reweighed and the mass of water lost over each trial calculated by subtracting the final chamber weight from the initial chamber weight. The wall thickness of the exposed area of the capsule piece was determined by marking the exposed piece of capsule wall, sectioning it using a freeze microtome, and then measuring the thickness of the section under a compound microscope. To control for water lost through the diffusion chamber itself, I also measured weight loss from intact cuvettes (i.e., no diffusion hole) that were filled with water and sealed with Parafilm, as described above. The water loss per unit time in these control chambers was then subtracted from experimental chambers.

Measures of diffusion coefficients can be artificially lowered by a boundary layer around the diffusion chamber which retards the movement of water vapor. To estimate the boundary-layer effects, I measured the diffusion rate of water from chambers filled with a 4% agar solution (i.e., effectively a free water surface with no impediment to diffusion). This was accomplished by placing a piece of tape over the diffusion hole, and then filling cuvettes with 4 ml of 4% agar solution. Once the agar had set, the tape was removed, the Plexiglas plate attached, and the chamber sealed with Parafilm and placed within a desiccator (37% rh @ 18°C) for ≈ 1 h. Chambers were then removed and reweighed. The resistance of the boundary layer to water vapor movement was subtracted from the resistance of the capsule wall plus boundary layer (as measured above) to give the resistance of the capsule wall itself.

b) Estimates of the mass transfer coefficient

The mass transfer coefficient was estimated for capsules from Grappler Inlet, Ross Islet, and Kirby Point by using measures of desiccation rates of capsules exposed to moving air (see above). Using these data, I was able to solve equation (4) for h_m by assuming that capsules were opened-ended cylinders, whose dimensions were based on the capsule body length and chamber width of real capsules. The wall thickness of these cylinders was estimated to be the mean wall thickness @ 70% along the length of the capsule chamber for each specific population of capsules examined (see Table 4-1). The diffusion gradient of water vapor intensity across the capsule wall was determined by calculating the vapor pressure difference between the evaporating surface and the environment for each experimental trial, using recorded values of temperature and humidity. I assumed that the vapor pressure density at the capsule surface was equivalent to that of saturated air at ambient temperature, corrected for the salinity (32‰) of seawater (see Appendix 2). This ignored any difference between the temperature of the ambient air and capsule surface associated with evaporative cooling, even though this difference may be substantial (see Bell, in press). Air movement around the capsule was also characterized by means of a dimensionless term, the Reynolds number (Re). This value is dependent on wind speed, the length of the object parallel to flow, and the kinematic viscosity of air (see Appendix 2).

Estimates of the diffusion coefficient of capsule walls to water flux and the magnitude of the mass transfer coefficients were verified empirically using cylindrical agar egg capsules. Agar models have been used in previous studies to estimate the rate of water loss from free water surfaces of other objects (e.g., whole amphibians and reptiles: Spotila and Berman, 1976; bird eggs: Spotila et al., 1981), because they allow the boundary layer resistance ($r_b = 1/h_m$) to be separated from the total resistance of an object to water loss ($r_b + r_w$). To compare water loss from *Nucella* egg capsules to a free water surface of similar size and shape, I constructed cylindrical agar models by pouring a warm agar solution (4% by weight) into a number of plastic cylindrical templates (6 x 3 mm). An artificial "stalk" was provided by embedding a plastic stick (2 mm in length) into the agar; this facilitated handling models and allowed them to be positioned easily on the Plexiglas stand within the wind tunnel. Once the agar had set, models were removed from their template and then kept within a desiccator (100% rh @ 20°C) until needed.

Preliminary trials in static and moving air were conducted, as described above, to determine the duration over which the rate of water loss from these models was linear. Based on these results, agar models were desiccated for a period of 5 minutes under wind velocities ranging from 0 - 1.5 m/s. A short time period was essential to ensure that the agar surface did not dry out over this duration (see Spotila et al., 1981). Prior to each trial, agar models were weighed, and then individually assigned to the wind tunnel. Immediately following each trial, models were reweighed to determine the rate of water loss while in the wind tunnel.

c) Effect of capsule wall thickness on desiccation rate

Once measures of the diffusion coefficient and mass transfer coefficients were determined, these values were used to model the effects of varying wall thickness on the rate of water loss from *N. emarginata* egg capsules. To do this, I modeled environmental conditions at a constant humidity and temperature of 80% rh and 18°C, and simply varied wind speed by altering the magnitude of the mass transfer coefficient. The size of these cylindrical capsules was also kept at a constant 6 x 3 mm. Because previous results indicated that few embryos survived > 80% water loss from the capsule chamber, I also modeled the amount of time taken for thick- and thin-walled capsules to lose this amount of water under varying wind conditions.

Microclimatic conditions associated with naturally-spawned capsules

Spawning sites: In August/September 1994, I conducted a field survey at Ross Islet, Wizard Rock, and Kirby Point, to characterize the areas selected by *N. emarginata* as spawning sites, given the range of microhabitats available at these locations. Because an algal covering can substantially ameliorate desiccation stress for understorey organisms (see Schonbeck and Norton, 1978), I examined the extent of algal canopy associated with spawning areas. To do this, I placed a 5-10 m long transect line parallel to the waterline, at a tidal height that intersected the vertical distribution of *N. emarginata* (Ross Islet: 2.4 m [above ELWS, chart datum]; Wizard Rock: 2.9 m; Kirby Point, 3.2 m). To determine the local availability of microhabitats for capsule deposition within the selected intertidal area, I placed a 0.1 x 0.1 m quadrat (divided into 25 x 0.004 m^2 squares) at intervals of either 0.25 or 0.5m along the transect line. Within each quadrat, I estimated the percent cover of any algal canopy (e.g., *Fucus gardneri, Mastocarpus papillatus*) covering the primary substrate. This algal cover was then removed from the quadrat, and collected for a measure of wet weight. The percent cover of all understorey turf algae (primarily *Cladophora*) was also estimated, and then removed from each quadrat. Once in the laboratory, algal samples were damp dried by squeezing them between paper towels and then weighed to 0.01 g.

To assess the frequency of microhabitats selected by spawning females, I searched for 20 groups of intact egg capsules within 0.25 m on either side of the transect line. Once a group of capsules was found, it was marked by attaching a piece of fluorescent flagging tape next to the capsule mass. The quadrat was then centered over each group of capsules, and the percent cover of algal canopy and understorey turf algae was estimated. Algal samples were removed from each quadrat for estimates of wet weight, as described above.

Microclimatic conditions: To compare the microclimatic conditions among different intertidal microhabitats, I recorded the relative humidity, temperature, and wind speed within areas differentially covered by algal canopy. Measurements were taken at Ross Islet and Wizard Rock during low tide on two warm summer afternoons; both sites were exposed to a prevailing westerly wind on these days. All measurements were taken after these microhabitats had been exposed to air for ≈ 4 - 6 h at each location. Quadrats (0.01 m^2) were positioned along a transect line within the vertical range of *N. emarginata* to sample an array of different microenvironments; each quadrat was categorized according to the percent cover of the algal canopy overlying the primary substratum. To measure humidity and temperature, I mounted a thermohygrometer probe on a stand above each quadrat and then gently lowered this under the algal canopy (when present) until the protective tip of the probe abutted the substratum. In this position, the sensor was ≈ 10 mm above the rock surface. The probe was then shaded from direct sunlight, and allowed to equilibrate for five minutes before temperature and humidity readings were taken (6 measurements at 10 second intervals). This probe was then removed and a hot-wire anemometer lowered into its place. The anemometer was positioned so that its protective tip abutted the substratum, and was aligned in the direction of the prevailing wind. Ten measurements were recorded at 10 second intervals at a height of ≈ 10 mm above the rock surface.

To examine how microclimatic conditions varied over the complete period of tidal emersion, I monitored air temperature, wind speed, and relative humidity within microhabitats associated with *Nucella* egg capsules at two locations: 1) a north-west facing vertical rock face at a wave sheltered location in Grappler Inlet, and 2) a westerly-facing rocky shoreline at an intermediate wave-exposed location at Ross Islet. These sites were chosen because both were frequently used as spawning sites by *N. emarginata* and could be monitored without the concern of unpredictable swells or wave-spray. The rock face at Grappler Inlet was primarily covered with barnacles (*Semibalanus cariosus*; *Balanus glandula*) and mussels (*Mytilus trossulus*), with little *Fucus* canopy present; capsules were usually deposited within bare areas within these mussel and barnacle patches. In contrast, the shoreline at Ross Islet was densely covered with *Fucus gardneri*. Measurements were made on three separate days in 1993: July 7 and 9 at Grappler Inlet, and September 12, at Ross Islet. To monitor the microclimatic changes following emersion, I mounted a thermohygrometer and anemometer probe at a predetermined location within 30 - 45 minutes following the recession of the tide; these probes were kept in place until 30 minutes prior to reimmersion by the incoming tide. Recordings were taken every 20 minutes (Temp/Humidity: 6 measurements at 10 second intervals; wind speed: 10 measurements at 10 second intervals). I also measured the humidity of the surrounding air (1 m off the substratum) at the same time using a sling psychrometer.

While measuring these environmental parameters, I determined the weight lost from capsules placed within specific microhabitats at these sites. These capsules had been collected from Ross Islets a few days prior to conducting these experiments. Capsules, as close in size as possible, were mounted in small holders made of Tygon tubing, and were arranged singly or in groups by altering the spacing of capsules within these small holders (Fig. 4-2). As soon as the hygrometer and anemometer probes had been set in place, capsules and their holders were positioned in microhabitats along a transect line. For measurements along the rock wall at Grappler Inlet, I tried to place these capsule holders within similar microhabitats to those that I was sampling for microclimatic conditions. At Ross Islet, however, because of the varied *Fucus* cover, I simply placed capsule holders in a range of microhabitats along the transect line and recorded the percentage cover of overlying algal canopy. Paired holders containing either single or grouped capsules were always placed within 10 - 20 mm of each other so that differences in desiccation rate associated with the grouping of these capsules could be compared. Once the incoming tide was about to rewet these capsules, they were placed within small dry plastic microcentrifuge vials and returned to the lab, where they were weighed immediately (weight after desiccation), and reimmersed in seawater for 24 h. Capsules were then reweighed (wet weight), dried in a desiccator (80% rh) to constant weight, and weighed again (dry weight). The percentage of capsule volume lost during emersion was calculated using equation (1). Because capsules may have lost weight during transportation back to the laboratory, I controlled for this by determining the percentage of weight change during transportation for a representative group of capsules.

Modeling water loss from thick- and thin walled capsules under field conditions

I used the microclimatic conditions recorded at Grappler Inlet on July 7 and 9, and Ross Islet on September 12 to model water loss from thick- and thin-walled cylindrical capsules under realistic field scenarios. To do this, capsules were modeled as cylinders, as before, using mass transfer coefficients derived from Ross Islets (see Results), and a wall thickness of either 52.6 or 79.1 µm (means of thin and thick-walled capsules, respectively). Because desiccation rates varied not only in response to environmental conditions, but also depending on the amount of water remaining in the capsule chamber (see Results), I had to incorporate this change in desiccation rate over time into the model. By determining the expected relationship for capsules drying at a linear rate versus the non-linear desiccation rate of real capsules (see Results), I was able to correct for the dependence of desiccation rate on the amount of water remaining within the capsule chamber.

Apart from differences in the thickness of capsule walls, *N. emarginata* capsules also vary substantially in size and shape among populations (Table 4-1). To examine the relative importance of site-differences in capsule size and shape versus capsule wall thickness, I modeled water loss from thick- and thin-walled cylindrical capsules with different surface area to volume ratios. In this model, capsules were exposed to microclimatic conditions recorded from Ross Islets on September 12, 1993 for a period of three h. I used dimensions of cylinders that spanned the range of capsule body lengths and chamber widths measured from real field-collected capsules of *N. emarginata*. Hence, capsules varied in surface area to volume (SA/VOL) ratios from 1.09 to 1.57, with capsules from Grappler Inlet representing the low extreme and capsules from Ross Islet the high extreme.

Results

Rates of water loss from *N. emarginata* egg capsules

a) Desiccation rates in still air

Preliminary measurements: Capsules from Ross Islets and Kirby Point dried to constant weight over 2 -3 h under ambient laboratory conditions (rh: 30 - 37%; T = 20 - 22.5°C) (Fig. 4-3a); variation among capsules in the time taken to desiccate completely was based largely on initial differences in capsule size. Desiccation rates were relatively constant over the initial stages of water loss, but then slowed considerably after capsules lost > 60% of their volatile water. A similar trend has been reported previously for

Nucella lapillus capsules (Bayne, 1968). This profile of water loss was identical among all capsules when capsules were standardized according to the time taken to lose half the capsule volume (Fig. 4-3b).

Among-population comparisons: The percentage of water lost per capsule over 50 minutes in still air (37% rh @ 20°C) differed among populations, with capsules from Grappler Inlet, Ross Islet, and Kirby Point losing, on average, 36.4 ± 1.49, 41.6 ± 1.23, and 41.1 ± 1.15% (mean ± SE) of their volatile water, respectively (n = 15 capsules / population). These average site-differences were largely related to initial differences in capsule size, since the percentage of water lost per capsule was inversely related to the amount of volatile water (Fig. 4-4; Table 4-2). Interestingly, however, Grappler Inlet capsules lost a significantly higher percentage of their volatile water per unit size over this period relative to capsules from Kirby Point and Ross Islet (Fig. 4-4; Table 4-2a).

The rate of water lost per capsule also depended on the surface area of the capsule body for each population (Fig. 4-5; Table 4-2b). Comparisons of these relationships among populations revealed that Grappler Inlet capsules lost water at a higher rate per unit surface area than capsules from both other sites (Table 4-2b). This was not expected *a priori* since Grappler capsules had the thickest walls (see Table 4-1).

b) Desiccation rates in moving air

Preliminary measurements: Moving air greatly increased the rate of water loss from capsules under ambient laboratory conditions of temperature and humidity. Capsules exposed to wind speeds of 1.5 m/s lost > 80% of their volatile water over 30 minutes; desiccation rates at reduced wind speeds were substantially lower (Fig. 4-6a). Again, rates of water loss were relatively linear until capsules lost from 50 - 60% of their volatile water. When capsules were standardized according to the time taken to lose 50% of their volatile water, desiccation profiles were very similar (Fig. 4-6 b). This relationship was described by the quadratic function: $Y = 10.1508 (X - 3.1842)^2$, where y = % volatile water loss, and x = time / time to lose 50% volatile water. This function was used to model the rate of water loss from cylindrical egg capsules (see below), since the time taken to lose any percentage of the capsule volume could be estimated from a) the initial rate of water loss of an egg capsule, and b) the weight of volatile water in the capsule.

Among population comparisons: Based on results from preliminary trials, capsules were placed in the wind tunnel for a period of 15 minutes. This ensured that capsules did not lose > 60% of their capsule volume over this time period. The percentage of volatile water lost from capsules strongly depended on both wind speed (Fig. 4-7; Table 4-2c), and also on capsule size, as larger Grappler Inlet capsules lost significantly less water per given wind velocity than smaller capsules from Ross Islet and Kirby Point (Table 4-1, Table 4-2c). Because the temperature and humidity of the surrounding air were not controlled in this experiment, the rate of water loss per unit surface area of capsule was corrected for differences in environmental conditions by dividing by the gradient in water vapor density across the capsule wall. The resulting "conductance", measured in m/s, describes the ease with which water is lost from intact *N. emarginata* capsules and is equivalent to $1/r_t$, where r_t is the total resistance of the capsule wall and boundary layer to the diffusion of water. Comparisons of the conductance among capsules collected from Grappler Inlet, Ross Islet and Kirby Point illustrated that the loss of water from capsules increased logarithmically with Reynolds number for all sites (Fig. 4-8), although water was lost at a significantly faster rate from Ross Islet capsules than from Grappler Inlet and Kirby Point capsules (Table 4-2d). Interestingly, this difference among sites appeared to increase with increasing wind speed (Fig. 4-8).

Mortality of encapsulated embryos exposed to desiccation stress

Both early- and late-staged embryos of *Nucella emarginata* tolerated extensive water loss from the capsule chamber (Fig. 4-9). In fact, appreciable mortality of embryos was only evident among capsules exposed to 60 - 85% water loss. Collection and temperature controls also indicated that natural levels of mortality of encapsulated embryos were generally very low (< 5%; Collection controls, Fig. 4-9) and that embryonic mortality did not result from the extended incubation of embryos at 18°C (Temperature controls, Fig. 4-9).

Mortality of late-staged embryos was often associated with the collapse of the capsule walls as the chamber desiccated. Within capsules exposed to > 60% water loss, for instance, 39.5% of dead embryos (n = 261) were crushed so severely that their shell was in pieces and yolk had ruptured through the body wall. A large percentage of dead embryos (40.3%), including some that were crushed, also showed signs of obvious physiological stress, as noted by their purple coloration (see Spight 1977; Gallardo, 1979; Pechenik, 1982, 1983). Other embryos exhibited tissue necrosis, but no signs of purple coloration or shell damage (26.1%). Only 1.5% of dead embryos were represented by empty shells. Within capsules exposed to less than 60% water loss, however, the majority of dead embryos were represented by empty shells (53.8% of 13 embryos). Only one of thirteen embryos was crushed (7.7%), while the remaining embryos (38.5%) showed evidence of extensive tissue necrosis.

The survival of early-staged embryos declined less abruptly with increasing desiccation stress relative to late-stage embryos (Fig. 4-9). Although dead embryos at very young developmental stages were often hard to distinguish from the mass of deteriorating nurse eggs, most embryos could usually be identified by their remaining larval shells. Because of this uncertainty, however, embryos were not categorized according to the probable cause of mortality.

Models of water loss from egg capsules

a) Estimates of the diffusion coefficient of capsule wall material

The diffusion coefficient of the capsule wall to water molecules was estimated to be $2.36 \times 10^{-6} \pm 0.162 \times 10^{-6}$ m^2/s (mean ± SE; n = 7) for egg capsules of *N. emarginata* at a temperature of 18°C. This value was close to measures of the diffusion coefficient for both *Nucella lamellosa* and *Nucella canaliculata* capsules (data not shown) and approximately one-tenth of the diffusivity of water vapor in air (2.42×10^{-5} m^2/s at 20°C; Campbell, 1977). Although the magnitude of the diffusion coefficient is known to be slightly temperature dependent (e.g., water vapor in air varies from 2.2 to 2.49×10^{-5} m^2/s over a temperature range from 10 - 25°C; Campbell, 1977), in the desiccation models described below, diffusion coefficients for *N. emarginata* capsules were assumed to be constant over a range of temperatures from 10 - 22 °C.

Estimates of the diffusion coefficient of the capsule wall were confirmed using cylindrical agar capsules. The resistance of the capsule wall was calculated by subtracting the boundary layer resistance (estimated from cylindrical agar capsules) from the total resistance of the capsule wall and boundary layer to water diffusion (estimated from real egg capsules) (Fig. 4-10). From this, the diffusion coefficient of the capsule wall was determined by dividing the capsule wall resistance by the capsule wall thickness, estimated as the average wall thickness of capsules sampled from all three populations (69.8 μm). The diffusion coefficient of capsule walls measured in this manner varied from 1.76×10^{-6} to 4.06×10^{-6} m^2/s over a range of Reynolds numbers from 30 to 300. The change in diffusion coefficients with wind speed was unexpected, but may have resulted from an underestimation of boundary layer resistances at low wind speeds by the agar models (see Spotila et al., 1981). Nevertheless, this range of values was reasonably close to values estimated previously using diffusion chambers (see above).

b) Estimates of the mass transfer coefficient

The magnitude of the mass transfer coefficient, describing the conductance of water through the boundary layer of air surrounding *N. emarginata* capsules, depended strongly on wind velocity (Fig. 4-11). For all three sites, increased Reynolds number resulted in higher mass transfer coefficients, thus illustrating the inverse relation between boundary layer thickness and wind speed. Ross Islet capsules, however, had significantly lower mass transfer coefficients for a given Re compared to Kirby Point and Grappler Inlet capsules (Table 4-2e). Reassuringly, the mass transfer coefficients for cylindrical agar capsules were similar in magnitude to those measured for real egg capsules of *N. emarginata* (Fig. 4-11, dotted line).

To model the effects of capsule wall thickness and wind speed on rates of water loss from *N. emarginata* capsules, I chose one site-specific relationship for h_m *vs.* Re. Since the relationships for Grappler Inlet and Kirby Point capsules were not significantly different, I modeled the relationship for thick-walled capsules from Grappler Inlet. The choice of relationships, however, did not have any qualitative effect on the outcome of the results.

c) Effect of capsule wall thickness on desiccation rate

Interestingly, increased wall thickness had very little effect on modeled rates of water loss from *N. emarginata* egg capsules at low wind speeds, but a substantially larger effect as wind velocity was increased (Fig. 4-12). This agreed well with observed differences in water loss among thin-walled Ross Islet capsules and thick-walled capsules from Grappler Inlet and Kirby Point at low and high wind speeds (Fig. 4-8). Over a range of wind speeds from 0.01 - 1.29 m/s, thick-walled capsules required an increasing amount of time to lose 80% of the capsule volume relative to thin-walled capsules (Fig. 4-13). This percent difference in time varied as much as 20% between thick- and thin-walled capsules at a wind speed of 1.29 m/s (Fig. 4-13).

Microclimatic conditions associated with naturally-spawned capsules

Female *N. emarginata* at Ross Islet and Kirby Point preferentially selected areas for spawning that had an extensive algal cover. Although, on average, the algal canopy only covered 62 and 70% of the substratum at these two sites (Fig. 4-14), snails spawned capsules in areas with > 90% algal cover. In contrast, understorey turf algae was not associated with increased capsule production; in fact at Ross Islet, snails appeared to avoid spawning in areas with extensive *Cladophora* cover (Fig. 4-14). Measurements of microclimatic conditions in these areas confirmed that sites chosen by snails for spawning also provided egg capsules with the greatest protection from desiccation stress. At both Ross Islet and Wizard Rock, an increase in the percent cover of algae resulted in substantially lower wind speeds and higher relative humidities (Fig. 4-15). Temperatures also tended to decline with increasing cover of *Fucus* and *Mastocarpus*. Results from the wave-exposed Kirby Point site, however, differed substantially from Wizard Rock and

Ross Islet. At this site algal cover was virtually non-existent. Instead, snails spawned capsules in either the empty tests of the barnacle, *Semibalanus cariosus,* or squeezed next to other intertidal organisms, such as aggregations of goose-neck barnacles (*Pollicipes polymerus*) (see Chapter 5).

Environmental conditions within the vicinity of capsules changed considerably over a 7 - 8 h period of emersion (Fig. 4-16, 17). Measurements at Grappler Inlet were made on early morning low tides on July 7 and July 9, 1993 (low tide @ 9.29 am and 10.35 am, respectively). Although both days were warm and sunny, and air temperatures increased steadily over the duration of emersion, capsules were exposed to relatively more extreme conditions on July 9. Humidities dropped below 80% rh for only 1 - 2 h on July 7 versus 4 - 5 h on July 9. Wind velocities were also generally higher on July 9. Differences among days in temperature, humidity and wind speed were reflected in the amount of weight lost from capsules placed in microhabitats along the same tidal height as those sampled for microclimatic conditions (Fig. 4-18). Percent water lost from capsules ranged from 0.9 - 15.1% on July 7, and 0.5 - 55.5% on July 9, respectively. The percentage of weight lost per capsule also depended on the grouping of capsules with respect to each other. Capsules placed in isolated arrangements consistently lost more water than aggregated capsules, although this effect was not quite significant for July 9 (Fig. 4-18). Only a small percentage of volatile water was lost by control capsules during transport back to the laboratory, although on July 7 this represented a substantial amount relative to experimental capsules.

Microclimatic readings were also recorded within a quadrat with 52% algal cover at Ross Islet on a sunny afternoon in September 12, 1993 (low tide @ 3.59 pm). Environmental conditions were quite severe on this day relative to measurements recorded at Grappler Inlet (Fig. 4-19). Temperatures varied from 19 to 21°C for most of the period of emersion and dropped only during the late afternoon when the intertidal zone became shaded by nearby tree cover. Humidities also fell below 80% rh for over 4 h, and wind velocities reached up to 0.28 m/s. Capsules placed in microhabitats ranging from 48 - 84% cover over the same period of emersion lost a varying amount of water (Fig. 4-20). Those capsules lacking an extensive cover of algae lost from 75 - 100% of their volatile water during 8 h of emersion. In contrast, those capsules under an 85% cover of algae lost only very little of their capsule volume during this period. Interestingly, under these microclimatic conditions, there was no obvious difference in the percentage of weight lost among isolated and aggregated capsules.

Modeling water loss from thick- and thin walled capsules under field conditions

Models of water loss from thick- and thin-walled cylindrical capsules revealed only relatively small differences in the percentage of volatile water lost after 7 - 8 h of emersion (July: 41.5% vs. 44.1%; July 9: 73.0% vs. 77.9%; Sept. 12: 92.4% vs. 96.3%; for thick- and thin-walled capsules respectively; Fig. 4-21). Under the most extreme conditions of September 12, thick-walled capsules would have required only ≈ 20 minutes longer to lose 80% of their initial water compared to thin-walled capsules (Fig. 4-21). Although the estimated percentage of water lost from these cylindrical capsules was higher than that recorded for actual capsules under field conditions for all three days (see Fig. 4-18, 4-20), the qualitative difference in the amount of water lost among days was similar.

Differences in capsule size and shape among sites had a large effect on the rate of water loss from the capsule chamber relative to site-differences in capsule wall thickness (Fig. 4-22). Thin-walled capsules modeled under microclimatic conditions recorded at Ross Islet on Sept. 12, 1993, for example, lost from 30.9 to 38.2% of their capsule volume over 3 h depending on their SA/VOL ratio; those capsules with high SA/VOL ratios lost up to 7.1% more of their chamber volume compared to those with low ratios. In contrast, the amount of water lost by thick- and thin-walled capsules over the same range of capsule sizes and shapes differed by only 3.3 - 3.6% (Fig. 4-22). Thick capsule walls did provide those capsules with large SA/VOL ratios with slightly greater protection from desiccation than those with low SA/VOL ratios, however. Under the microclimatic conditions modeled here, therefore, variation in capsule size and shape among populations had almost twice as great an effect on the rate of water loss from cylindrical *N. emarginata* capsules than the among-site differences in capsule wall thickness.

Discussion

Like egg capsules of other neogastropod species (e.g., Bayne, 1968; Pechenik, 1978), the capsule walls of *Nucella emarginata* are not impervious to water loss. Indeed, given the rate at which water is lost from egg capsules of *N. emarginata* under laboratory conditions, and the frequency and duration of exposure of these capsules to air, desiccation stress has the potential to be a very important source of mortality for encapsulated embryos. Embryonic survival during periods of tidal emersion may thus depend primarily on the deposition of these structures within humid, wind-sheltered microhabitats, rather than with properties of the capsule wall itself. Nevertheless, since *Nucella* capsule walls have a 10-fold lower diffusivity to water vapor than air, they do provide embryos with some protection from desiccation. Also, given the tolerance of embryos to substantial water loss from the capsule chamber and associated fluxes in salinity, encapsulated embryos may be well adapted to withstanding desiccation stress within a broad range of environmental conditions.

Rates of water loss from *Nucella emarginata* capsules

Although *Nucella emarginata* egg capsules desiccated rapidly under laboratory conditions, capsules exhibited a very characteristic decline in the rate of water loss over time. Dehydration occurred at a relatively constant rate until > 50% of the water was lost from the capsule chamber; the rate of desiccation then proceeded to decrease exponentially over time (Fig. 4-6). Interestingly, a similar profile of water loss over time has also been described for intertidal algae (e.g., Kristensen 1968, Schonbeck and Norton, 1979; Jones and Norton, 1979; Dromgoole, 1980; Bell, in press), as well as for eggs of other marine and terrestrial gastropods (Bayne, 1968). This decline in water conductance may likely have many causes (see Dromgoole, 1980), associated both with a change in the humidity gradient surrounding the capsule, as well as a change in the resistance of the capsule wall to water loss.

One of the most probable causes of this change in water conductance over time may be due to an increase in salt concentration within the capsule chamber resulting from the loss of water (e.g., Slayter, 1967; Foster, 1971; Jones and Norton, 1979). An increase in solute concentration can lower the humidity gradient between the capsule and its environment and thus lower the "desiccation potential" across the capsule wall. A loss of 50% of the volatile water, for instance, doubles the concentration of salts, and hence, reduces the humidity gradient across the capsule wall by 3.7% relative to pure water (see Appendix 2). Increased salt concentration progressively decreases the desiccation potential as less and less water remains in the capsule chamber. Because extensive water loss from the capsule chamber also deforms the capsule walls, however, the continued loss of water may also be affected by a) capsule wall stiffness, and b) changes in capsule size and shape. For instance, Daniel and Pechenik (pers. comm.) have suggested that as a capsule buckles with increasing dehydration, and the walls become stretched, the stiffness of the capsule walls will resist inward deformation (see Feder et al., 1982). In this way, the stiffness of the capsule wall can reduce the rate of water loss. Changes in capsule size and shape with increasing dehydration may also affect the surface area exposed for water loss and the thickness of the boundary layer. Since the boundary layer thickness can limit the rate of water loss from *N. emarginata* egg capsules (see below), capsule shape changes may have substantial effects on rates of water loss. The relative impact of each of these factors on rates of water loss from *Nucella* egg capsules remains to be determined, however.

Despite the frequency and duration of exposure of *Nucella emarginata* capsules to air, their walls provide little resistance to water loss relative to the egg coverings of other organisms. Although quantitative estimates of resistances (r_W) for the egg coverings of marine intertidal invertebrates are rare, estimates for egg shells and cases of terrestrial organisms are common. Bird eggs under conditions of moving air exhibit resistances of ≈ 38,500 s/m (Spotila et al., 1981, assuming a shell of 0.3 mm thick), compared to values of 22.4 to 33.6 s/m for *Nucella* egg capsules (determined from measures of diffusion coefficients, assuming a wall thickness of 52.6 to 79.1 µm). Insect egg membranes are also extremely resistant to water loss (McFarlane, 1966; 1970) and their permeability to water may be among the lowest recorded for any animal membrane (Edney, 1977). For instance, eggs of some species can withstand incubation at low humidities (e.g., < 5% rh) for days to weeks without any net water loss (Biemont et al., 1981). In contrast, the parchment shelled eggs of lizards and snakes have resistances to water loss that are considerably lower than those of bird and insect eggs: the shell resistance of snake and lizard eggs range from 210 to 526 s/m (@ 24 - 26 °C) under conditions of still air (Ackerman et al., 1985). Nevertheless, these values are still appreciably higher than those measured for *Nucella* capsules. Hence, in fully terrestrial environments, egg coverings appear to provide developing embryos with much more substantial protection from the rigors of desiccation than do the capsule walls of *N. emarginata*. This may not be true for the shelled eggs of terrestrial gastropods, however (see Bayne, 1968; Riddle, 1983).

The rate of water loss from encapsulated embryos depends both on the resistance of the capsule wall (or shell) to water movement and on the resistance of the surrounding boundary layer. Because the resistance to water flux of bird egg shells ranges from 200 to 685 times greater than the resistance of the boundary layer, a decrease in boundary layer thickness associated with increasing wind speed has negligible effects on water loss from avian eggs (Tracy and Sotherland, 1979; Spotila et al., 1981). This is in significant contrast to capsules of reptiles where the egg shell and boundary layer resistances are usually within the same order of magnitude (Ackerman et al., 1985), and thus, desiccation rates are very sensitive to air movement. In the present study, boundary layer resistances for *Nucella emarginata* capsules were estimated to range from 32 to > 70 s/m over a range of wind speeds of 0 - 1.5 m/s (Fig. 4-10), compared to capsule wall resistances of 22 to 34 s/m. At low wind speeds, therefore, where boundary layers are thick, rates of water loss from reptile egg shells and *N. emarginata* egg capsules, are governed primarily by boundary layer conditions, rather by the properties of the egg shell or egg case itself.

Interestingly, the same may also be true for water loss from intertidal algae (Jones and Norton, 1979).

Since capsules of *N. emarginata* are relatively permeable to water transport, and rates of water loss are substantially affected by exposure to wind, it is perhaps not surprising that many intertidal gastropods appear to show a strong preference for spawning their eggs within cool, damp microhabitats (e.g., Anderson, 1962; Emlen, 1966; Spight, 1977; Pechenik, 1978; Gallardo, 1979). For instance, *Nassarius obsoletus* deposit their egg capsules within the holdfasts of *Fucus spp.*, where encapsulated embryos experience substantially higher survival compared to other available habitats less protected by algal cover (Pechenik, 1978). Likewise, *Nucella* spp. also tend to spawn their capsules in areas associated with a low potential for water loss (Emlen, 1966; Spight, 1977; Gallardo, 1979). In the present study, wave-sheltered populations of *Nucella emarginata* exhibited a strong preference to spawn within a dense algal canopy where wind velocities and humidities were substantially lower than in the surrounding air. At more wave-exposed sites, such as Kirby Point, where little algal canopy is generally present (see Menge, 1978a), snails spawned capsules within empty barnacle tests of *Semibalanus cariosus* or squeezed next to aggregations of goose-neck barnacles (*Pollicipes polymerus*) (see specific details in Chapter 5). Although capsules were exposed to direct sunlight in these microhabitats, they appeared to remain relatively humid during emersion; few capsules were ever seen buckling in response to water loss. Hence, the deposition of egg capsules within protective microhabitats by female *Nucella emarginata* appears to compensate for the low resistance of capsule walls to water loss.

Adaptations of gastropod spawn to desiccation stress

Surprisingly, intertidal gastropod egg capsules do not appear to be better at protecting developing embryos from desiccation stress than capsules produced by subtidal gastropods. Bayne (1968) found a relatively similar rate of water loss per unit surface area from intertidal egg capsules of *Nucella lapillus* versus shallow-water egg masses of *Aplysia punctata* and *Lymnaea stagnalis*. Likewise, Pechenik (1978) determined that capsules of *Nassarius obsoletus* did not differ significantly in desiccation rate compared to capsules produced by their subtidal congener *Nassarius trivittatus*. Nevertheless, since both studies measured desiccation rates in either still or slow-moving air, their results should be interpreted with caution (see Ramsay, 1935). Unless desiccation rates are compared under conditions of fast-moving air, estimates of water loss may largely reflect boundary layer resistances rather than resistances of the capsule wall or jelly mass to water loss. Hence, definitive comparisons of the resistances of intertidal and subtidal egg capsules are still needed.

Thicker capsule walls nevertheless provide developing embryos with some means of increased protection from desiccation stress. The thick-walled capsules of *N. emarginata* collected from Grappler Inlet and Kirby Point exhibited lower rates of water loss per unit surface area than thin-walled capsules from Ross Islet under conditions of fast moving air (Fig. 4-8). In relatively still air, however, the converse was true: thick-walled capsules from Grappler Inlet dried at a faster rate per unit surface area than capsules from either Ross Islet and Kirby Point (Fig. 4-5). Although these results may seem contradictory, they are intuitive when considering the insulating nature of the boundary layer. In still air, the thickness of the boundary layer, which depends on capsule size and shape, greatly limits the desiccation rate. As wind strips away the boundary layer from an egg capsule, however, the thickness of the capsule wall becomes the rate-limiting factor. Simple models of water loss from cylindrical egg capsules of fixed size and shape have helped to verify these results by illustrating the increasingly important effect of wall thickness on desiccation rates at higher wind speeds (Fig. 4-12, 4-13). The selective advantage of being enclosed within thick versus thin-walled capsules, therefore, appears to depend on the type of microclimatic conditions that capsules experience under field conditions.

For capsules deposited within dense algal canopies, wind speeds are rarely high enough for thicker capsule walls to provide developing embryos of *N. emarginata* with significantly greater protection from desiccation. Desiccation models of thick- and thin-walled cylindrical egg capsules exposed to field conditions revealed only relatively small differences in the percentage of water lost over a 7 - 8 h period for three warm, windy days (Fig. 4-21). Since capsules are often spawned in groups rather than as isolated capsules (see Fig. 5-6 in Chapter 5), and aggregated capsules tend to lose water at a slower rate than isolated capsules (e.g. Fig. 4-18), microclimatic conditions experienced by capsules in the field may be further mollified by the spatial arrangement of capsules. Nevertheless, before the adaptive benefits of thick-walled capsules can be completely discounted, rates of water loss should also be modeled for thick- and thin-walled capsules within microhabitats lacking an algal canopy.

Interestingly, the increased thickness of protective barriers to water loss may also occur among other intertidal organisms exposed to desiccation stress. The cell wall thickness of fucoid algae, for instance, increases with tidal height (Zaneveld, 1937) and wave-exposure (Kristensen, 1968); plants with thicker cells walls also have substantially lower rates of desiccation under laboratory conditions. Although the interpretation of these results has been disputed (see Kristensen, 1968; Dromgoole, 1980), these associations nevertheless remain intriguing. If thicker barriers to water loss are a common response to increased desiccation stress among intertidal organisms, then perhaps the advantages of enclosing embryos behind thicker-walled barriers may indeed be significant under certain field conditions.

To investigate the effect of the capsule wall on rates of water loss from *Nucella emarginata* egg capsules in this study, I have downplayed the importance of capsule size and shape. Nevertheless, these attributes of *Nucella* egg capsules can clearly have a very important, albeit more predictable, effect on rates of water loss. Vermeij (1978), for instance, has summarized some common changes in the size and shape of intertidal invertebrates associated with an increased ability to tolerate heat and desiccation stress in high intertidal environments. Likewise, Dromgoole (1980) has argued that surface area to volume ratios are of primary importance in determining the ability of intertidal algae to survive extended periods of emersion. Comparisons of water loss from thick- and thin-walled cylinders, based on dimensions of field-collected capsules of *N. emarginata,* indicated that extreme differences in capsule SA/VOL ratios had almost twice the effect in reducing the percent water lost from capsules compared to differences in wall thickness (Fig. 4-22). Hence, variation in capsule size and shape may have substantially more effect on the ability of embryos to survive severe desiccation stress than among-population differences in capsule wall thickness. Given the fact that capsules with the lowest SA/VOL ratios and the thickest capsule walls are present at Grappler Inlet, however, it remains possible that changes in capsule shape and wall thickness may be common responses to increased desiccation stress at this site. Likewise, since Ross Islet capsules have the highest SA/VOL ratios and the thinnest walls, this may reflect the low potential for exposure to desiccation stress at this location. Nevertheless, although low SA/VOL ratios invariably will increase the ability of encapsulated embryos to endure periods of emersion, it is not clear to what extent these differences in capsule size and shape reflect adaptive responses to environmental conditions or are simply the product of ecophenotypic variation in snail form (see Chapter 2). Thus, further work is necessary to model the effects of other parameters of gastropod egg capsules on rates of water loss, and to examine the genetic and environmental basis of these differences in capsule form.

Tolerance of embryos to desiccation stress

Although the deposition of capsules within sheltered microhabitats may provide *Nucella emarginata* embryos with substantial protection from desiccation, their survival within the high intertidal zone undoubtedly relates to their ability to tolerate losses of up to 80% of the capsule volume. The ability of gastropod embryos to survive substantial dehydration appears to be well established in the literature. For instance, eggs of the terrestrial slug *Limax flavus* can withstand up to 85% weight loss before death (Carmicheal and Rivers, 1932), while eggs of the *Agriolimax* are able to survive ≈ 60 - 80% weight loss (Bayne, 1969). Advanced embryos of *Agriolimax,* like *Nucella emarginata,* also tolerate desiccation better than earlier stage embryos (see Bayne 1969), although this trend differs among other species (e.g., Carmicheal and Rivers, 1932). The tolerances of encapsulated embryos to water loss may not be as extreme as they first appear, however. Because water lost from the capsule can come from either the capsular case, the capsular fluid, or the embryos themselves, the percentage of water lost from individual embryos may be considerably less than 60 - 80%. For instance, in desiccating eggs of *Limax flavus*, a loss of 65% from the whole egg corresponded to a weight loss of only 35 - 40% from the embryos themselves (Carmicheal and Rivers, 1932). Hence, embryos are presumably buffered to some degree by the intracapsular fluid bathing them within the capsule chamber.

The ability of embryos to tolerate extensive water loss from the capsule chamber implies an underlying ability to withstand tremendous fluxes in osmotic concentration. Water loss increases solute concentration in both animals and plants (Slayter, 1967). For instance, the mortality of intertidal limpets to desiccation stress may be attributed entirely to the concentration of internal fluids resulting from evaporative water loss (Wolcott, 1973). Mortality of intertidal barnacles due to desiccation stress is also associated with a substantial increase in the solute concentration of the blood (Foster, 1971). Unfortunately, however, little is known about the tolerances of *N. emarginata* embryos to high salinity stress. Encapsulated embryos of *Nucella* are generally very tolerant of low salinity stress, although their survival also depends on the rate of salinity change, and may also vary according to embryonic stage (Pechenik, 1982; 1983). Since there is little evidence to indicate that the properties of egg capsule walls vary among subtidal and intertidal environments (e.g., Pechenik, 1978), therefore, it would be interesting to compare the tolerances of encapsulated embryos among these habitats, to determine if the ability of embryos to survive large osmotic fluxes is associated with an increased probability of exposure to desiccation.

Future considerations

Because desiccation depends largely on three environmental variables, temperature, wind speed, and humidity, it has not been a simple factor to assess under intertidal conditions. More often than not, desiccation stress and the resistances and tolerances of organisms to dehydration have been assessed qualitatively by measuring the rates of water loss from algal species or benthic invertebrates under artificial field and laboratory conditions. This has usually been done with little or no regard to a) the microclimatic conditions that organisms are actually exposed to, or b) the resistances of organisms and their boundary layer to water loss. As I have shown in the present study, examinations of water loss from organisms (or capsules) under artificial conditions (e.g., still air) and a poor understanding of the magnitude of the various resistances to water loss, can lead to very different interpretations of the functional benefits of specific morphologies. Also, without such information, predictive models of the "performance" of specific morphologies based on the conditions that might be experienced over a tidal, lunar and annual cycle, are impossible. Given the tremendous range of conditions experienced by intertidal organisms, such models (e.g., Madsen and Maberly 1990; Bell, in press) may be the only way to determine the adaptive benefits of different morphologies in response to desiccation stress. Thus, the approach taken in the present study, although obviously simplistic in design, will hopefully lead to further models incorporating other aspects of capsule form, and other environmental variables, into long term models of embryonic survival under natural field conditions.

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Table 4-1. Characteristics of *N. emarginata* capsules collected from three sites in Barkley Sound, British Columbia. Capsule dimensions represent the mean values for ten clutches of capsules (n = 10) sampled from each population in 1991. Wall thickness values are the mean values for 20 clutches of capsules (n = 20) sampled from each population during 1990 and 1991.

	Mean ± S.E.					
Site	Capsule Body Length (mm)	Chamber Width (mm)	Volume† (µl)	Surface Area† (mm^2)	SA / Vol.	Wall Thickness (µm)
Grappler Inlet	7.20 ± 0.250	3.29 ± 0.060	41.09 ± 2.319	54.31 ± 2.036	1.33 ± 0.025	79.1 ± 1.69
Ross Islet	5.81 ± 0.142	2.82 ± 0.058	24.44 ± 1.380	38.35 ± 1.435	1.58 ± 0.030	52.6 ± 1.70
Kirby Point	5.77 ± 0.179	3.00 ± 0.065	27.58 ± 1.760	41.55 ± 1.761	1.52 ± 0.032	77.9 ± 2.29

† Capsule volumes and surface areas were calculated using the capsule body length and chamber width. See text for details.

Table 4-2. Regression equations and summary ANCOVA statistics for *Nucella emarginata* capsules desiccating under conditions of still and moving air. All slopes were significant at $P < 0.001$.

a) Log (% Water Lost) versus Log (Volatile Water, in g) in still air (see Fig. 4-4)

					Regression Equation:		
Site	Y-variable	X-variable	r^2	n	Slope (SE)	Intercept (SE)	Adjusted Mean†
Grappler	Lg (% Water Lost)	Lg (Vol. Water)	0.974	15	-0.538 (0.0244)	0.725 (0.0379)	1.603 (0.0026)^a
Ross Islet	Lg (% Water Lost)	Lg (Vol. Water)	0.984	15	-0.570 (0.0203)	0.659 (0.0341)	1.591 (0.0024)^b
Kirby Pt	Lg (% Water Lost)	Lg (Vol. Water)	0.961	15	-0.533 (0.0298)	0.718 (0.0500)	1.589 (0.0024)^b
			Significance‡:		0.603		0.001

b) Desiccation rate (in mg/min) versus Surface area (in mm^2) in still air (see Fig. 4-5)

					Regression Equation:		
Site	Y-variable	X-variable	r^2	n	Slope (SE)	Intercept (SE)	Adjusted Mean†
Grappler	Desicc. rate	Surface area	0.900	15	0.00285 (0.00026)	0.05728 (0.01389)	0.18897 (0.001834)^a
Ross Islet	Desicc. rate	Surface area	0.907	15	0.00273 (0.00024)	0.05836 (0.01036)	0.18370 (0.001681)^b
Kirby Pt	Desicc. rate	Surface area	0.890	15	0.00258 (0.00025)	0.06154 (0.01095)	0.18036 (0.001644)^b
			Significance‡:		0.730		0.008

c) Log (% Water Lost) versus Log (Wind Velocity, in m/s) (see Fig. 4-7)

Site	Y-variable	X-variable	r^2	n	Regression Equation:		Adjusted Mean†
					Slope (SE)	Elevation (SE)	
Grappler	Lg (% Water Lost)	Lg (Wind Vel.)	0.872	18	0.282 (0.0271)	1.595 (0.0073)	1.559 (0.0076)^{a}
Ross Islet	Lg (% Water Lost)	Lg (Wind Vel.)	0.925	16	0.348 (0.0266)	1.659 (0.0076)	1.614 (0.0080)^{b}
Kirby Pt	Lg (% Water Lost)	Lg (Wind Vel.)	0.864	15	0.341 (0.0318)	1.648 (0.0095)	1.604 (0.0072)^{b}
			Significance‡:		0.250		<0.001

d) Log (Conductance, in m/s) versus Log (Reynolds number) (see Fig. 4-8)

Site	Y-variable	X-variable	r^2	n	Regression Equation:		Adjusted Mean†
					Slope (SE)	Elevation (SE)	
Grappler	Lg (Conduct.)	Lg (Re)	0.911	19	0.310 (0.0235)	-2.411 (0.0507)	-1.752 (0.0059)^{a}
Ross Islet	Lg (Conduct.)	Lg (Re)	0.949	20	0.352 (0.0193)	-2.476 (0.0412)	-1.728 (0.0057)^{b}
Kirby Pt	Lg (Conduct.)	Lg (Re)	0.974	20	0.326 (0.0127)	-2.444 (0.0272)	-1.751 (0.0057)^{a}
			Significance‡:		0.307		<0.001

e) Log (Mass transfer coefficient, in m/s) versus Log (Reynolds number) (see Fig. 4-11)

Site	Y-variable	X-variable	r^2	n	Regression Equation Slope (SE)	Regression Equation Elevation (SE)	Adjusted Mean†
Grappler	Lg (h_m)	Lg (Re)	0.915	19	0.620 (0.0458)	-2.804 (0.0988)	-1.486 (0.0117)^a
Ross Islet	Lg (h_m)	Lg (Re)	0.941	20	0.530 (0.0312)	-2.721 (0.0668)	-1.595 (0.0114)^b
Kirby Pt.	Lg (h_m)	Lg (Re)	0.959	20	0.641 (0.0313)	-2.844 (0.0671)	-1.482 (0.0114)^a
Agar Caps	Lg (h_m)	Lg (Re)	0.967	20	0.310 (0.0135)	-2.279 (0.0277)	
				Significance‡:	0.097		<0.001

† Adjusted means were computed using a common slope for each site, since slopes did not differ significantly among sites. Adjusted means with the same letter are not significantly different from one another at $\alpha = 0.05$.

‡ Significance values for tests of parallel slopes and differences in adjusted means using ANCOVA.

Figure 4-1. The closed circuit, open jet wind tunnel used to measure water loss from *Nucella emarginata* egg capsules in the laboratory. Also shown are the anemometer and hygrometer probes mounted in position in the working section of the wind tunnel.

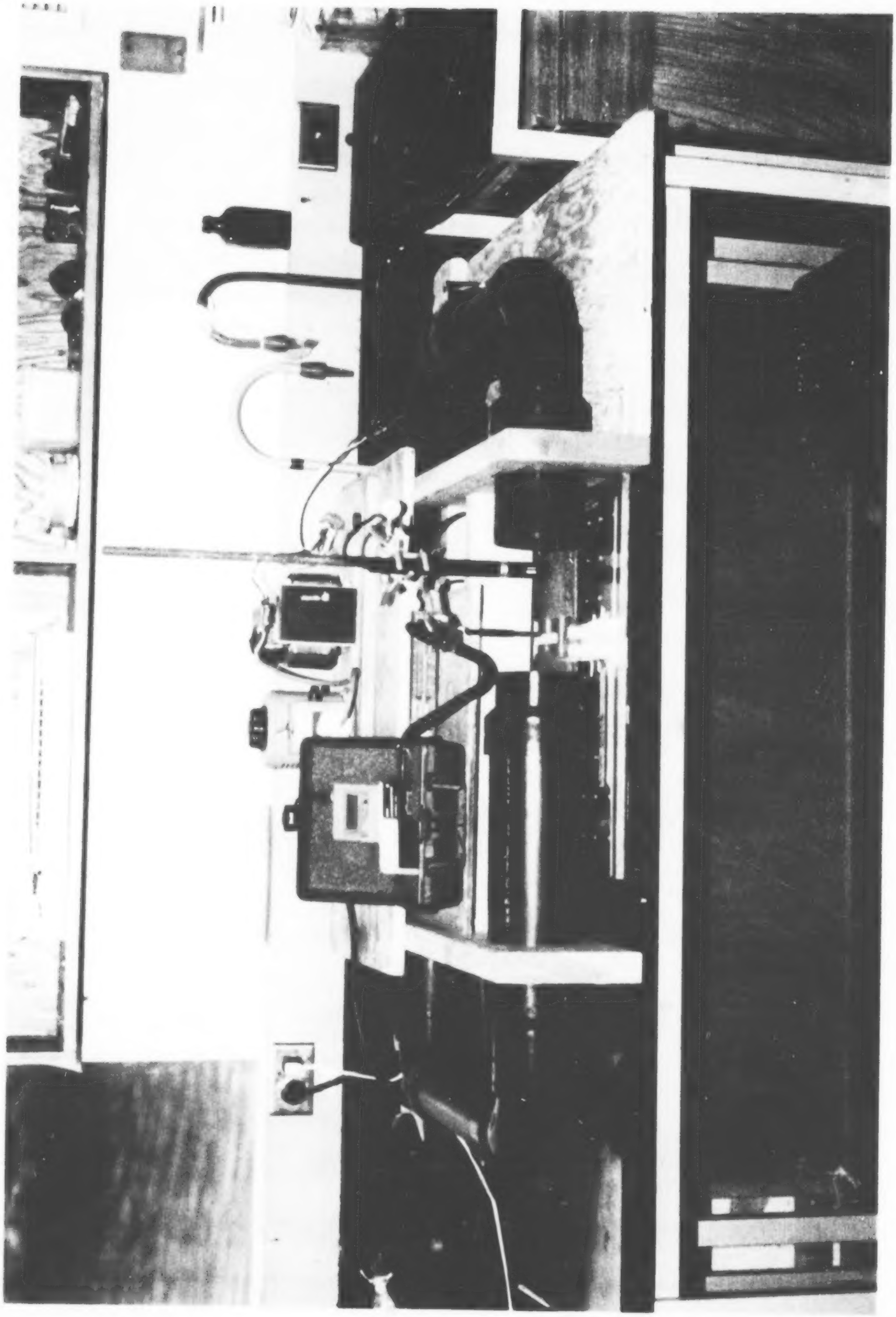
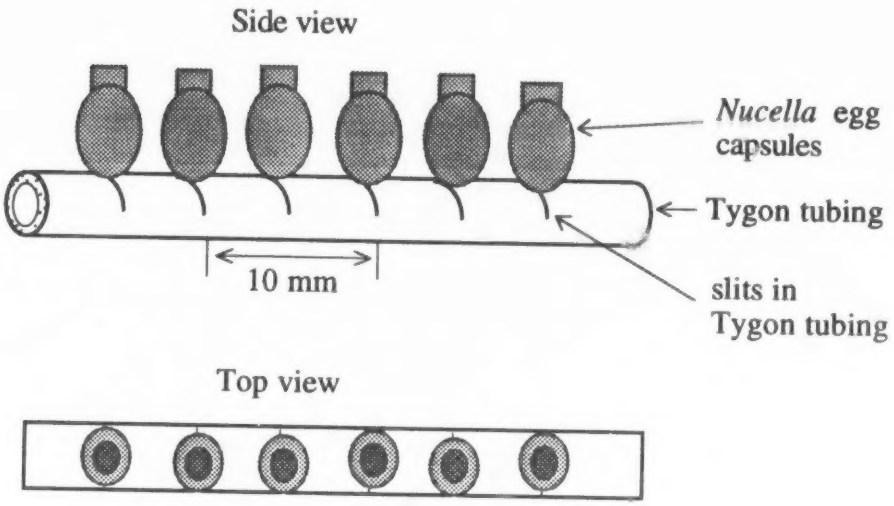


Figure 4-2. Two different arrangements of capsules mounted in their Tygon holders: a) "isolated capsules", and b) "aggregated capsules".

a) Isolated arrangement:


Side view
Nucella egg capsules
Tygon tubing
10 mm
slits in Tygon tubing
Top view

b) Aggregated arrangement:

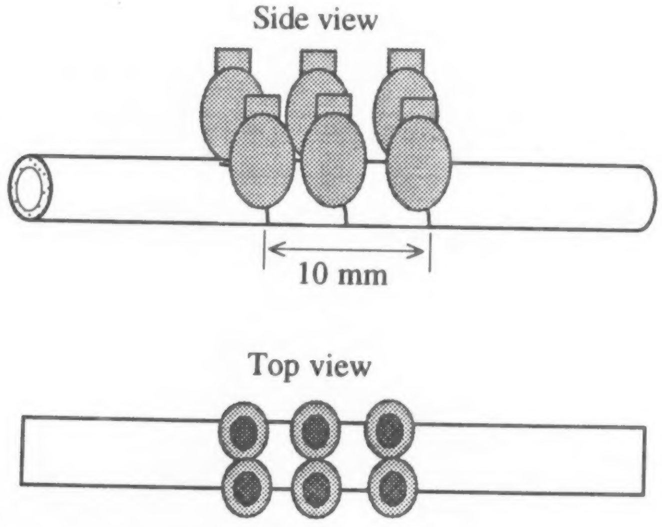

Side view
10 mm
Top view

Figure 4-3. a) Percent volatile water remaining within five *Nucella emarginata* capsules over time when exposed to laboratory conditions of still air. Temperatures and humidities varied during these experiments from 20 - 22.5°C, and 30 - 37% rh. b) Percent water remaining versus time for the same capsules in (a), when standardized for the amount of time taken to lose 50% of the volatile water from each capsule. The dotted line indicates the results one would expect if capsules dehydrated at a constant rate over time.

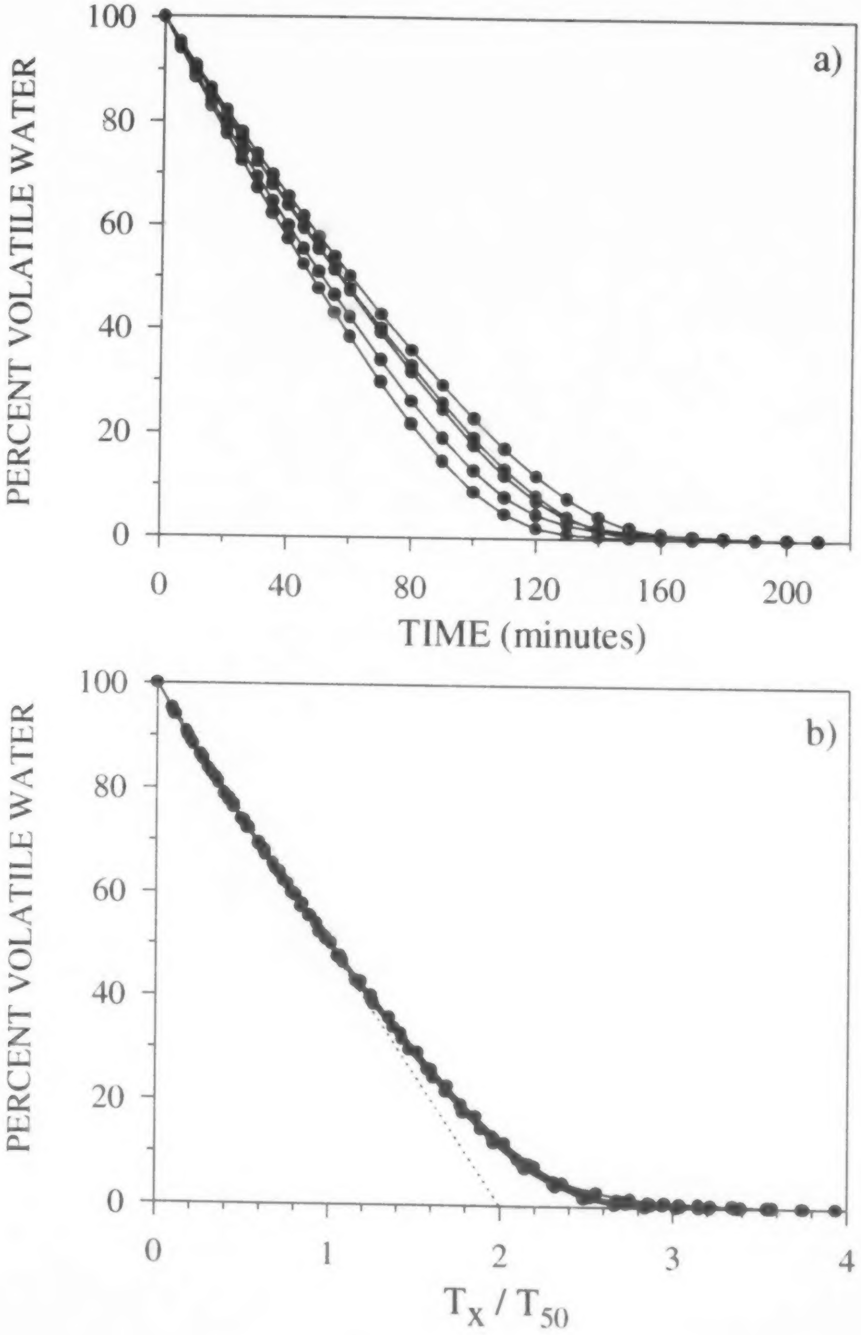


Figure 4-4. Logarithmic relationships between percent water loss and initial weight of volatile water for *Nucella emarginata* capsules from Grappler Inlet, Ross Islet, and Kirby Point populations. Capsules were kept in still air at a controlled temperature and humidity (37% rh @ 20°C) for a period of 50 minutes. Regression equations and ANCOVA statistics are given in Table 4-2a.

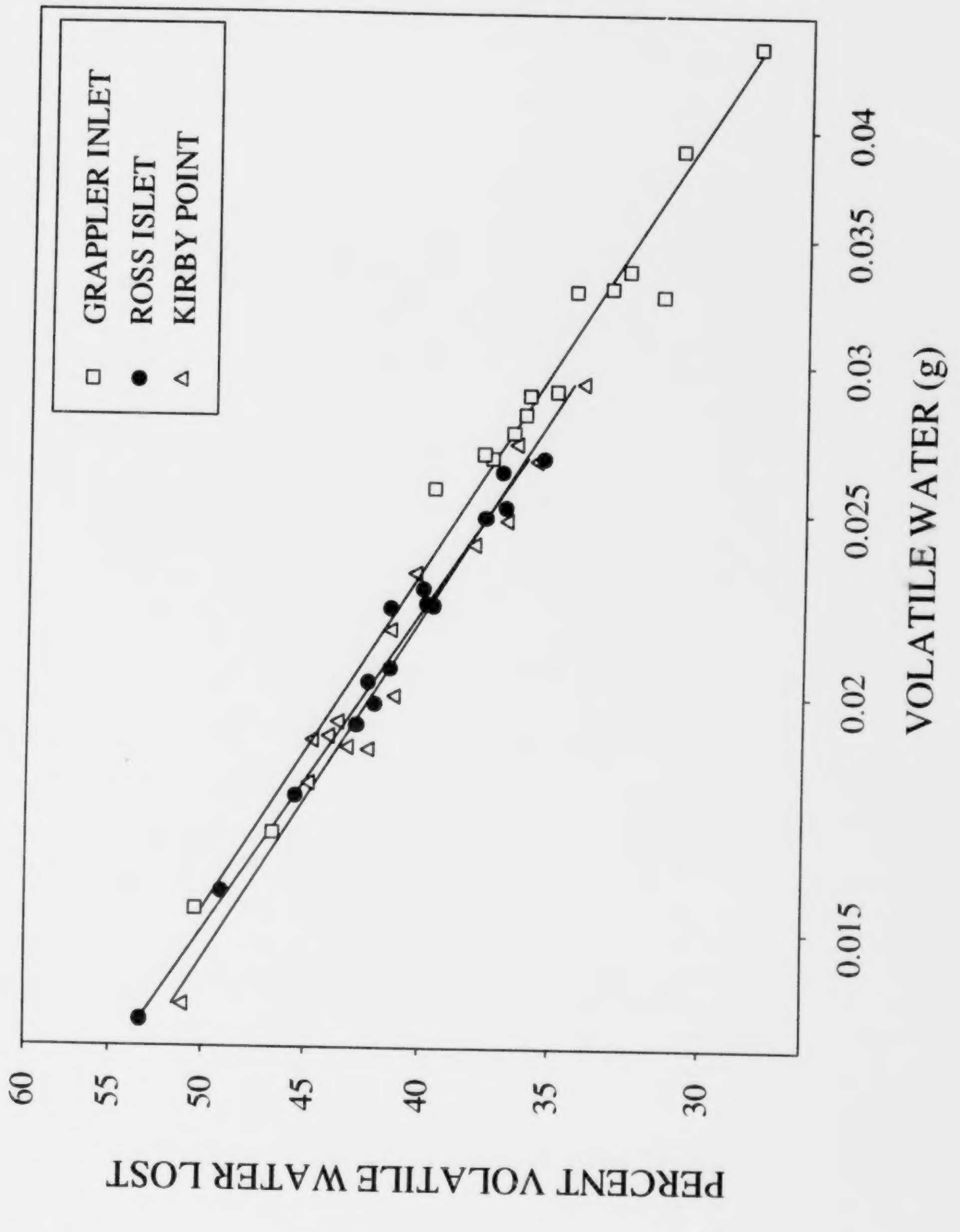

GRAPPLER INLET
ROSS ISLET
KIRBY POINT
60
55
50
45
40
35
30
PERCENT VOLATILE WATER LOST
0.015
0.02
0.025
0.03
0.035
0.04
VOLATILE WATER (g)

Figure 4-5. Relations between desiccation rate and surface area of the capsule body for capsules collected from Grappler Inlet, Ross Islet, and Kirby Point. Capsules were kept in still air at a controlled temperature and humidity (37% rh @ 20°C) for a period of 50 minutes. Surface area was calculated using the formula for a prolate ellipsoid (see Materials and Methods). See Table 4-2b for regression equations and ANCOVA statistics for each site.

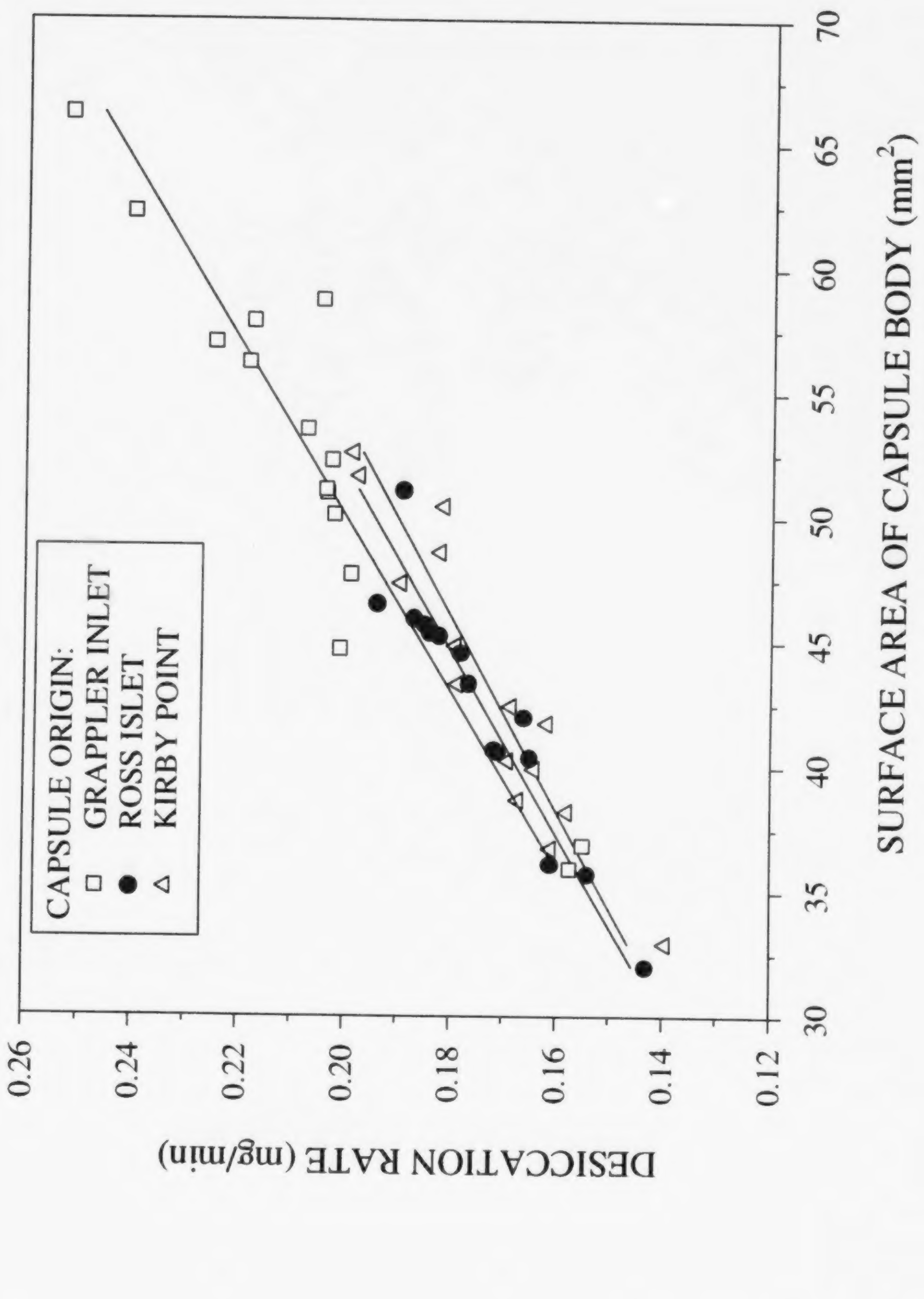

CAPSULE ORIGIN:
□ GRAPPLER INLET
● ROSS ISLET
△ KIRBY POINT
DESICCATION RATE (mg/min)
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
SURFACE AREA OF CAPSULE BODY (mm^2)
30
35
40
45
50
55
60
65
70

Figure 4-6. a) Percent volatile water remaining versus time for three replicate *N. emarginata* capsules exposed to wind velocities of 0.5 m/s, 1.0 m/s, or 1.5 m/s, and ambient conditions of temperature and humidity. b) Percent water remaining versus time for the same capsules in (a), when standardized for the amount of time taken to lose 50% of the volatile water from each capsule.

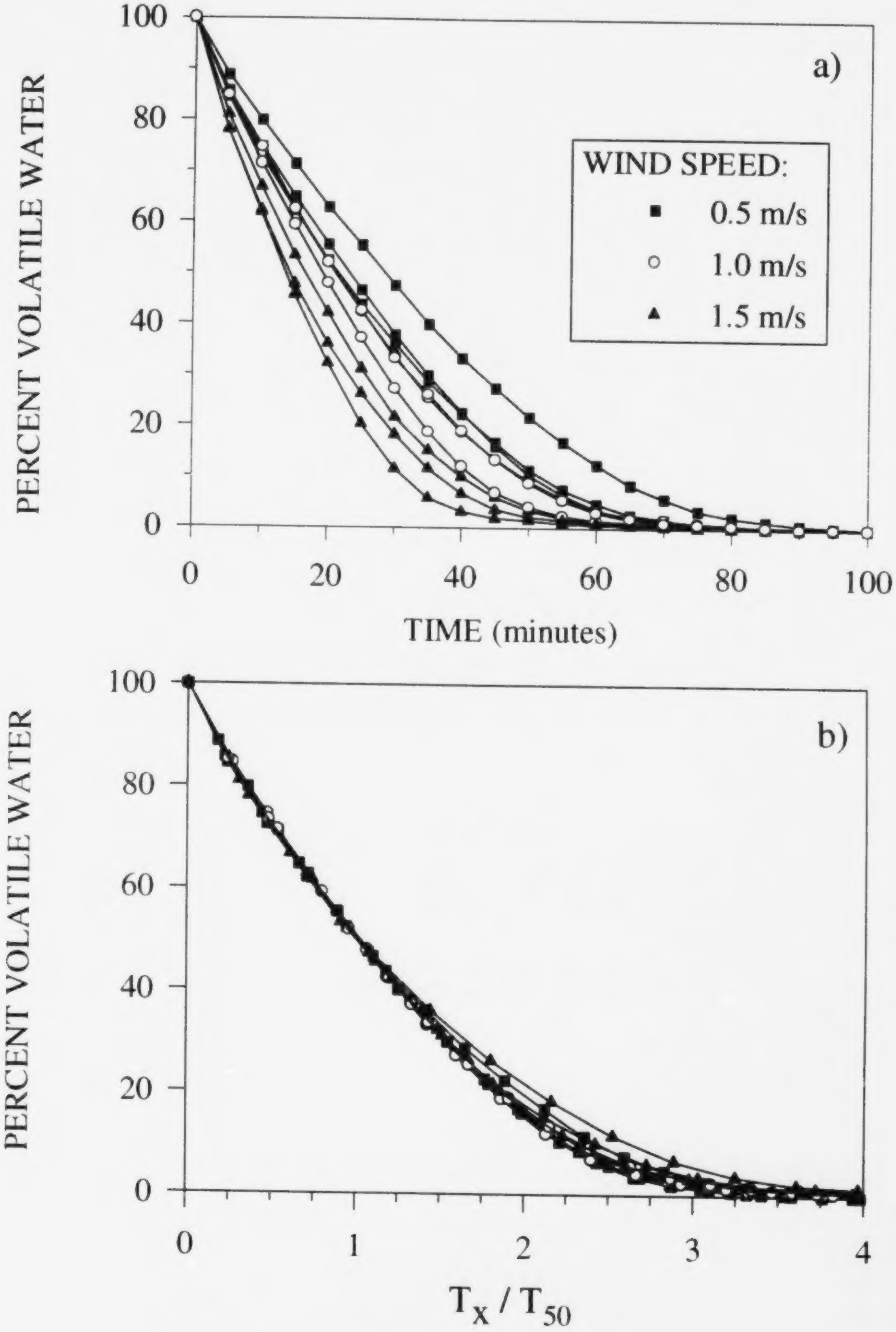


Figure 4-7. Logarithmic relationships between the percentage of volatile water lost versus wind speed, for capsules collected from Grappler Inlet, Ross Islet, and Kirby Point. Capsules were exposed to moving air for 15 minutes under ambient laboratory conditions. Regression equations and summary ANCOVA statistics are given in Table 4-2c.

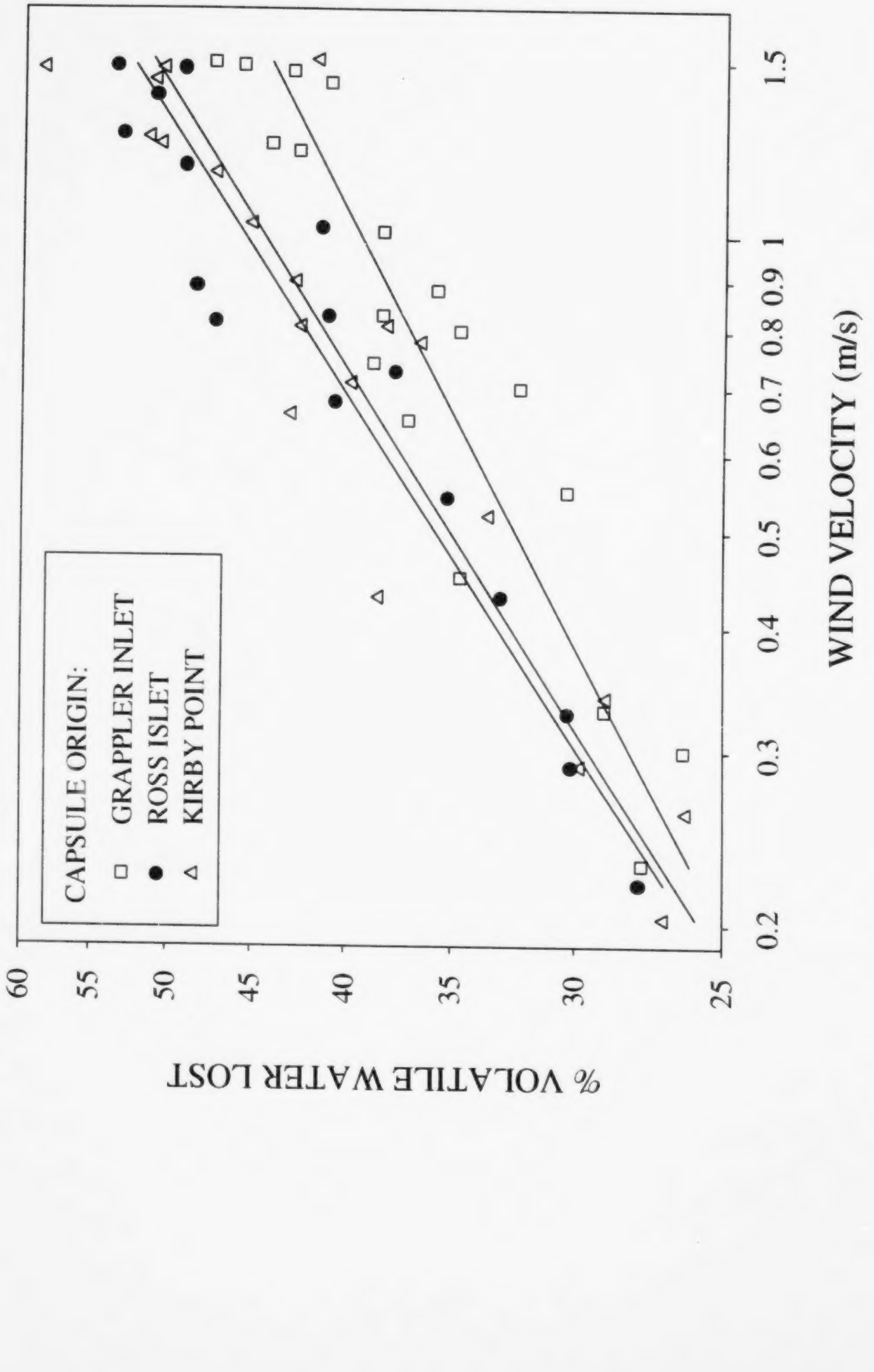

% VOLATILE WATER LOST
60
55
50
45
40
35
30
25
WIND VELOCITY (m/s)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.5
CAPSULE ORIGIN:
□ GRAPPLER INLET
● ROSS ISLET
△ KIRBY POINT

Figure 4-8. Logarithmic relationships between the conductance of capsule walls to water molecules and Reynolds number, for capsules collected from Grappler Inlet, Ross Islet, and Kirby Point. Capsules were tested within a wind tunnel for 15 minutes under ambient laboratory conditions. See Table 4-2d for regression equations and ANCOVA statistics.

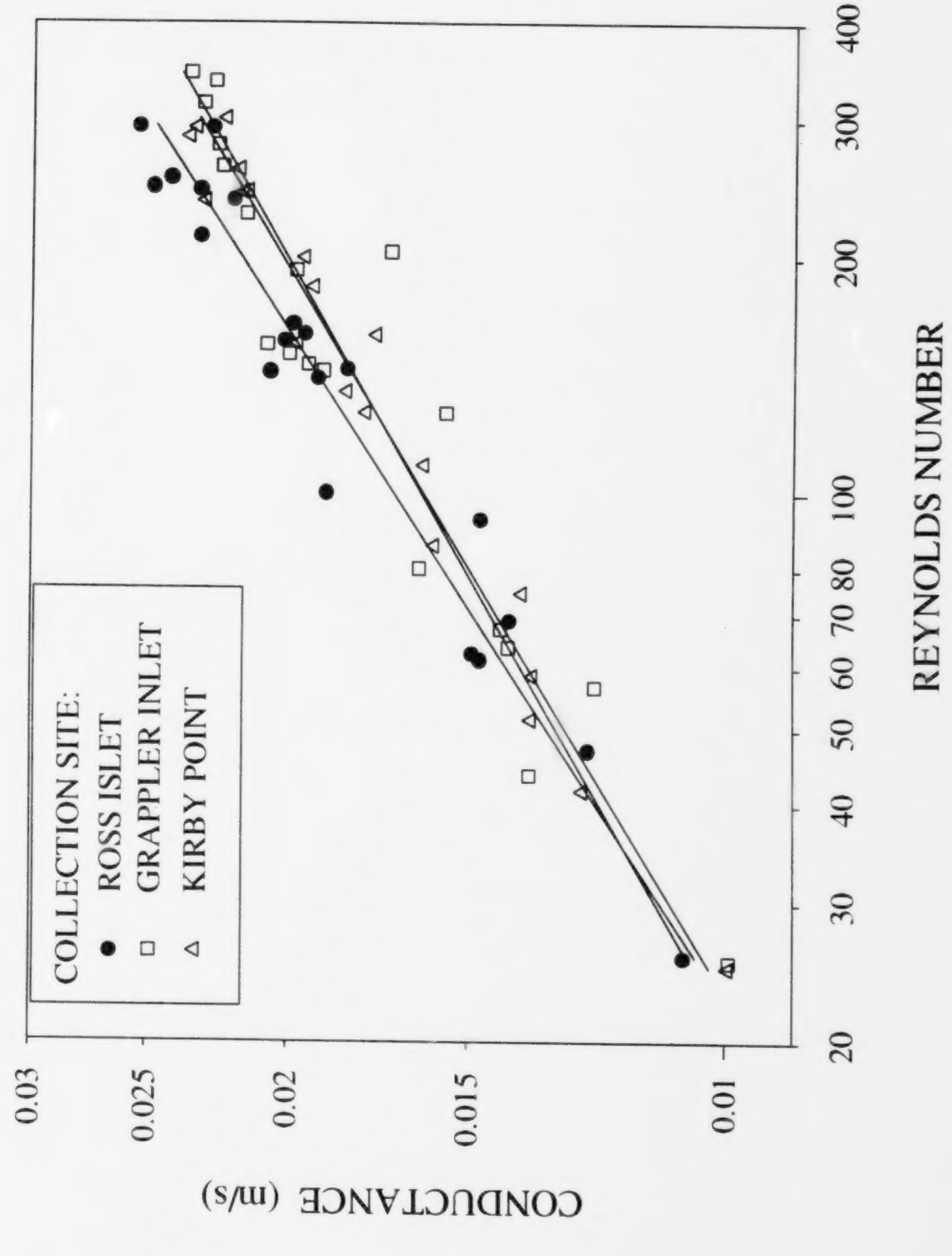

COLLECTION SITE:
● ROSS ISLET
□ GRAPPLER INLET
△ KIRBY POINT
CONDUCTANCE (m/s)
0.03
0.025
0.02
0.015
0.01
REYNOLDS NUMBER
20
30
40
50
60
70
80
100
200
300
400

Figure 4-9. Percentage of a) early-stage embryos and b) late-stage embryos within individual *N. emarginata* capsules surviving increasingly severe levels of water loss from the capsule chamber. Also shown is the mean (± SE) percentage of embryos alive in capsules collected from the field (Collection control), and the mean (± SE) percentage of embryos surviving 10 h exposure to 18°C at 100% rh (Temperature control). Sample sizes for capsules with early-stage embryos are: Experimental: 67 capsules; Collection control: 19 capsules; Temperature control: 10 capsules; and for capsules with late-stage embryos: Experimental: 70 capsules; Collection control: 20 capsules; Temperature control: 9 capsules. The results from one capsule in the Temperature control treatment (late-stage embryos) were not included in this figure. Although all embryos within this capsule were found dead, judging by their state of decay it appeared that these embryos were moribund prior to the experiment. In all nine other capsules, survival was > 80%, with a mean of 95.7%. If this capsule was included, the mean survival (± SE) was lowered to 86.2 ± 9.78%.

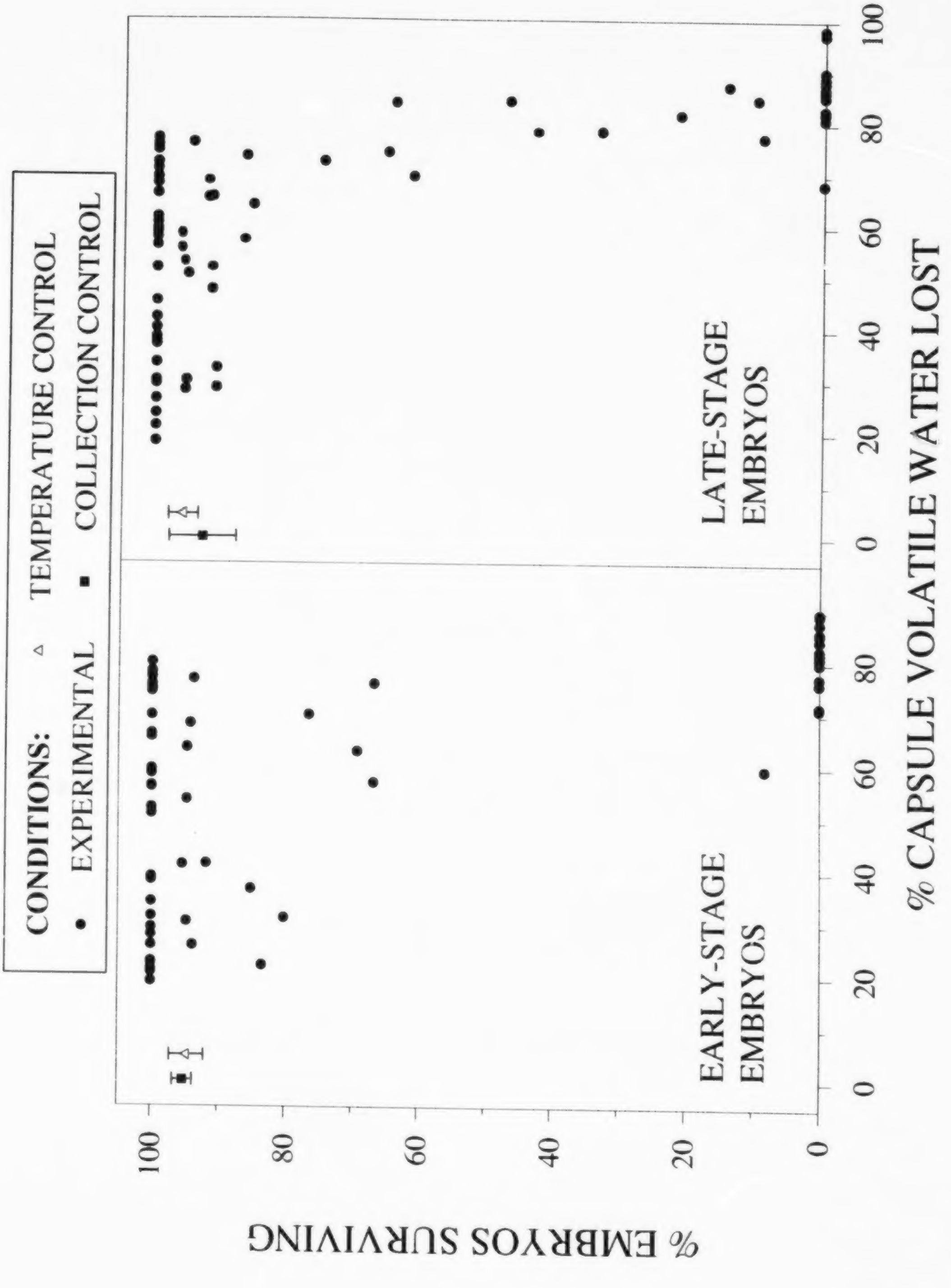

CONDITIONS:
● EXPERIMENTAL
△ TEMPERATURE CONTROL
■ COLLECTION CONTROL
EARLY-STAGE EMBRYOS
LATE-STAGE EMBRYOS
% EMBRYOS SURVIVING
% CAPSULE VOLATILE WATER LOST
0
20
40
60
80
100

Figure 4-10. Logarithmic relationships between a) the resistance of real *Nucella emarginata* egg capsules to water loss and Reynolds number, and b) the resistance of cylindrical agar egg capsules to water loss and Reynolds number. Experiments were conducted under ambient laboratory conditions. Data for *N. emarginata* capsules were pooled across sites. The difference between these two lines represents the resistance of the capsule wall to water loss. Regressions equations for these two relationships are: *N. emarginata* capsules: Log (Y) = -0.328 (0.0114) Log (X) + 2.509 (0.0245), r^2 = 0.936, n = 59; Agar capsules: Log (Y) = -0.310 (0.0136) Log (X) + 2.279 (0.0277), r^2 = 0.967, n = 20.

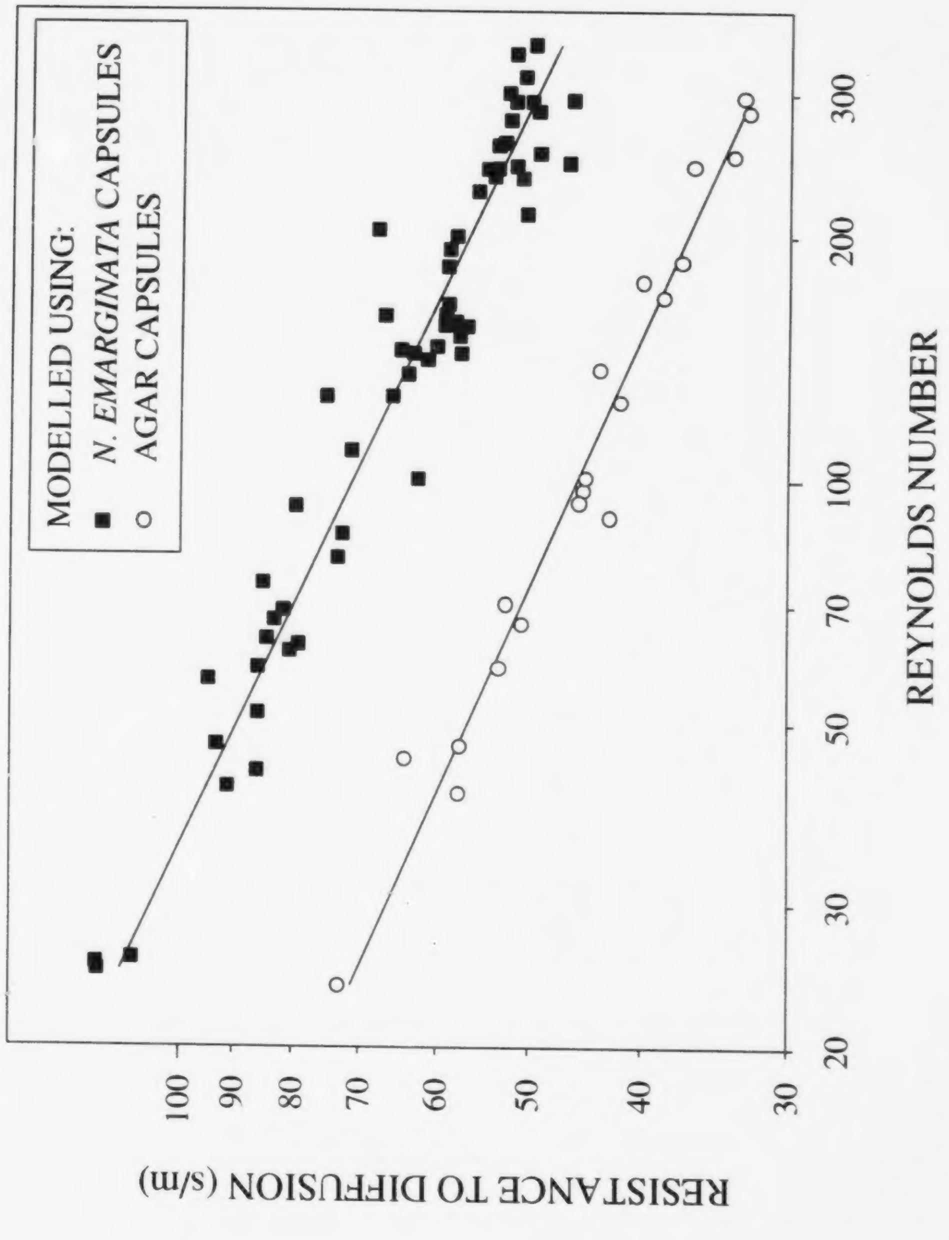

MODELLED USING:
■ N. EMARGINATA CAPSULES
○ AGAR CAPSULES
RESISTANCE TO DIFFUSION (s/m)
100
90
80
70
60
50
40
30
REYNOLDS NUMBER
20
30
50
70
100
200
300

Figure 4-11. Logarithmic relationships between the mass transfer coefficient and Reynolds number for capsules collected from Grappler Inlet, Ross Islet, and Kirby Point. Capsules were exposed to moving air for 15 minutes under ambient laboratory conditions. Also shown is the relationship determined for cylindrical agar capsules (dotted line). Regression equations and ANCOVA statistics are given in Table 4-2e.

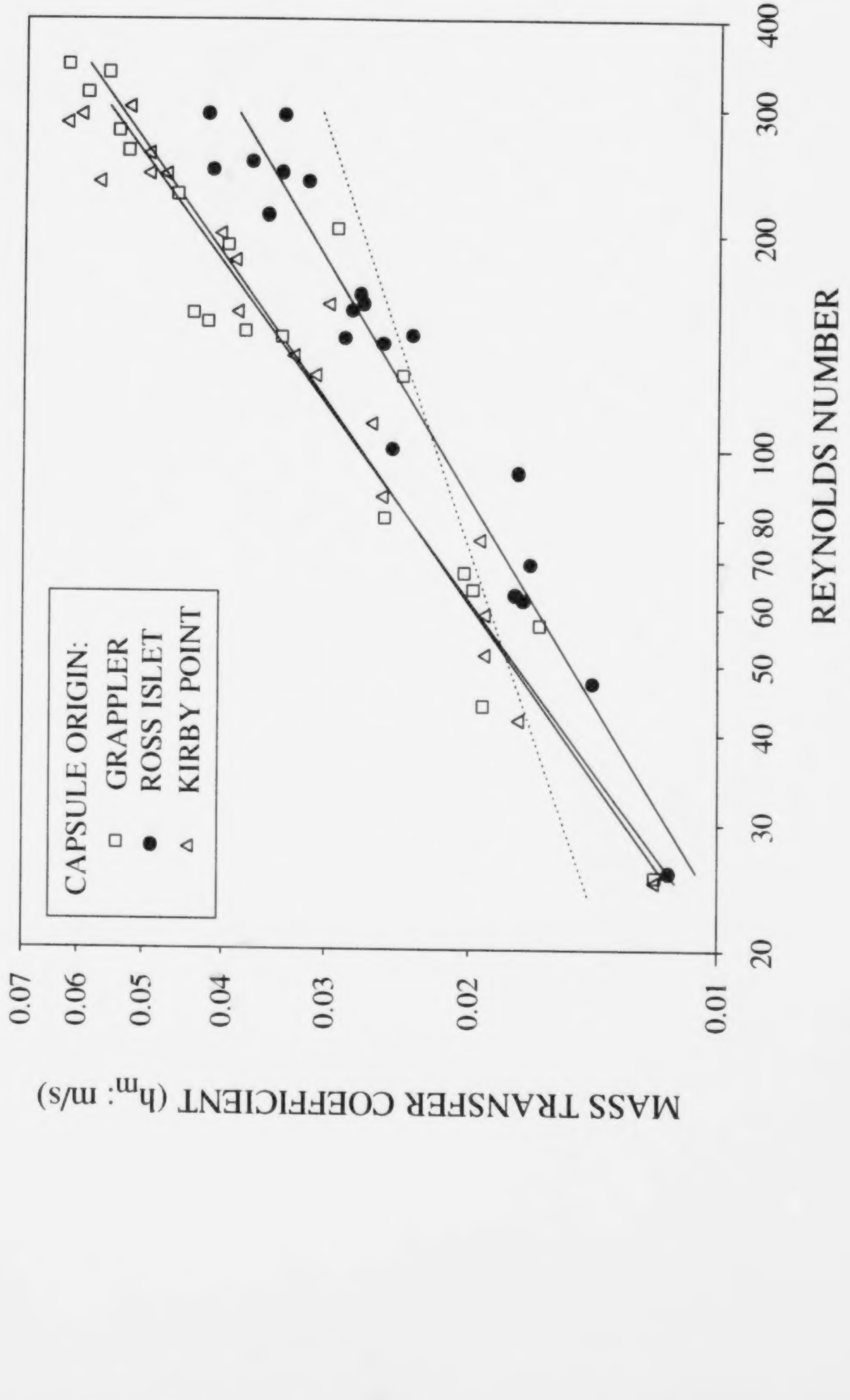

CAPSULE ORIGIN:
□ GRAPPLER
● ROSS ISLET
△ KIRBY POINT
MASS TRANSFER COEFFICIENT (h_m: m/s)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
REYNOLDS NUMBER
20
30
40
50
60
70
80
100
200
300
400

Figure 4-12. Effect of increasing wall thickness on the simulated rate of water loss from cylindrical egg capsules (6 mm x 3 mm) under variable wind conditions, but constant humidity and temperature (80% rh @ 18°C). Wall thickness is expressed as R_o/R_i, where a value of 1.0 indicates that there is no capsule wall. The two dotted vertical lines represent the average wall thickness of thin-walled capsules from Ross Islet (1.037) and thick-walled capsules from Grappler Inlet (1.057).

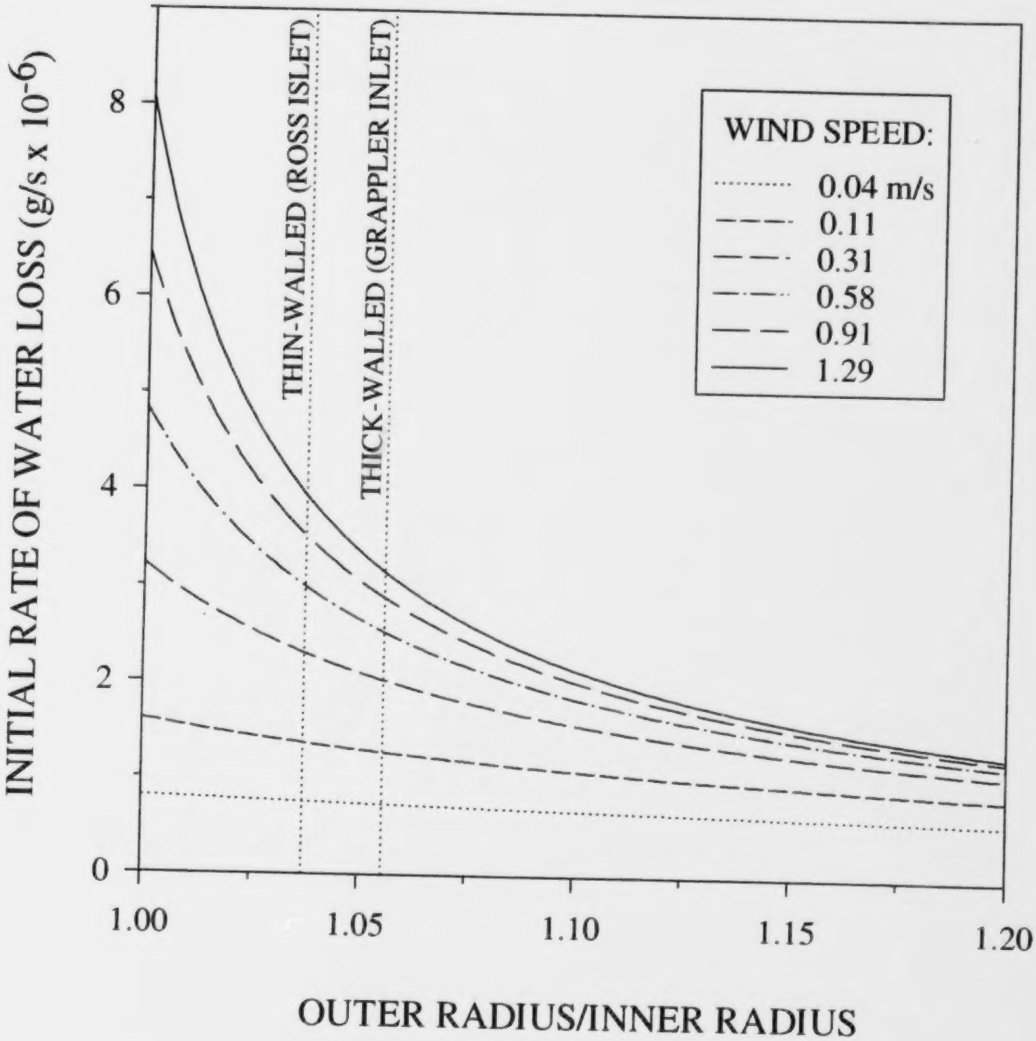


Figure 4-13. Simulated amount of time taken to lose 80% of the capsule volume from thick and thin-walled cylindrical capsules (6 mm x 3 mm) under variable wind conditions, but constant humidity and temperature (80% rh @ 18°C). Also shown (right vertical axis) is the percent increase in the time taken to lose this amount of water by thick-walled capsules relative to thin-walled capsules over this range of wind speeds.

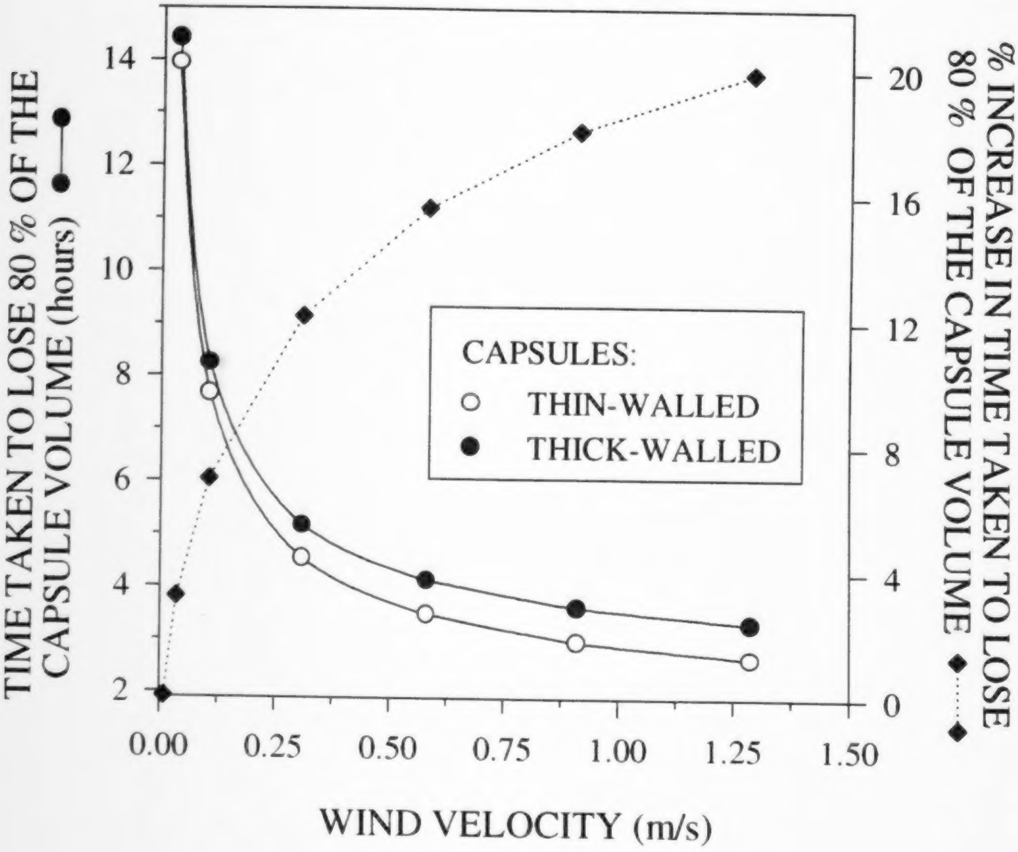


Figure 4-14. Average percent cover (± SE) of the primary substratum by algal canopy (primarily *Fucus*) and understorey turf algae (primarily *Cladophora*) in 20 "available" quadrats and 20 quadrats selected for spawning by *Nucella emarginata* at Ross Islet and Wizard Rock. Also shown on the right vertical axis is the wet weight of the algal canopy or turf algae in these quadrats. The percent cover of the *Fucus* canopy (arcsine transformed) differed significantly among available *vs.* selected microhabitats, but not among sites (Ross vs. Wizard): F(site) = 1.49; df = 1, 76; P = 0.23; F(microhabitat) = 24.01; df = 1, 76; P < 0.001; F(interaction) = 0.05; df = 1, 76; P = 0.81 (Two-way ANOVA). A similar comparison for the percent cover of algal turf showed a marginally significant site effect, and a significant microhabitat and interaction effect: F(site) = 3.79; df = 1, 76; P = 0.06; F(microhabitat) = 15.98; df = 1, 76; P < 0.001; F(interaction) = 9.63; df = 1, 76; P = 0.003 (Two-way ANOVA).

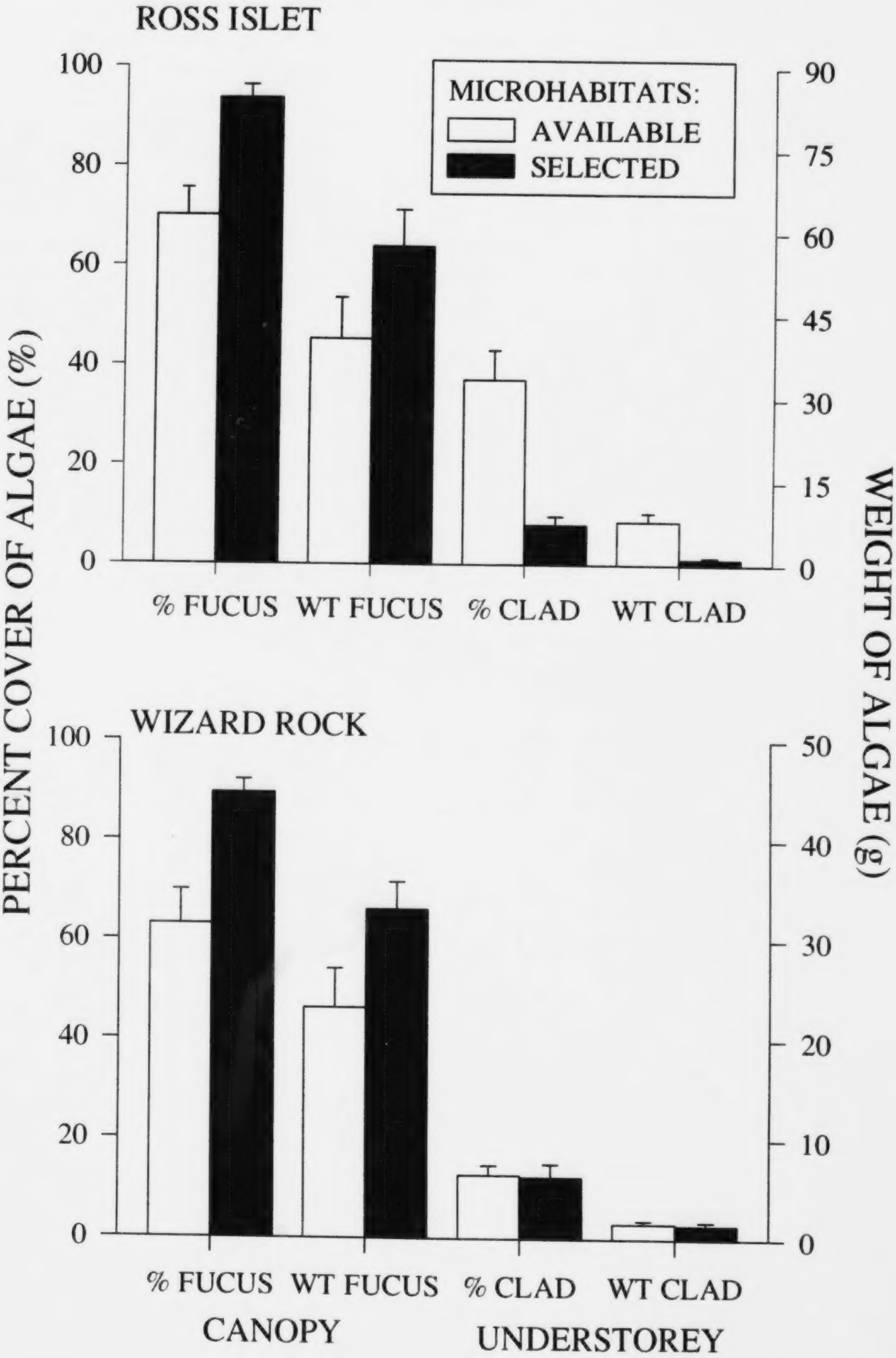

ROSS ISLET
MICROHABITATS:
AVAILABLE
SELECTED
100
80
60
40
20
0
90
75
60
45
30
15
0
% FUCUS
WT FUCUS
% CLAD
WT CLAD
WIZARD ROCK
100
80
60
40
20
0
50
40
30
20
10
0
% FUCUS
WT FUCUS
% CLAD
WT CLAD
CANOPY
UNDERSTOREY
PERCENT COVER OF ALGAE (%)
WEIGHT OF ALGAE (g)

Figure 4-15. Change in wind speed, temperature and relative humidity associated with increased algal cover within 0.1 m x 0.1 m quadrats at Ross Islet and Wizard Rock on two late summer afternoons (Ross: Sept. 13, 1993: 3 - 4.30 pm; Wizard: Sept. 1, 1994, 2 - 4 pm). Measurements were taken 4 - 6 h after quadrats had been emersed. Each temperature and humidity reading represents the average (± SE) of 6 measurements taken at 10 second intervals at a height of 10 mm above the substratum. Each wind speed measurement represents the average of 10 measurements taken at 10 second intervals at the same height above the substratum. Where error bars are not visible they are less than the diameter of the symbol. Curves were produced using a curve-fitting program in Sigmaplot (SPW, Version: 1.0; Jandel Scientific).

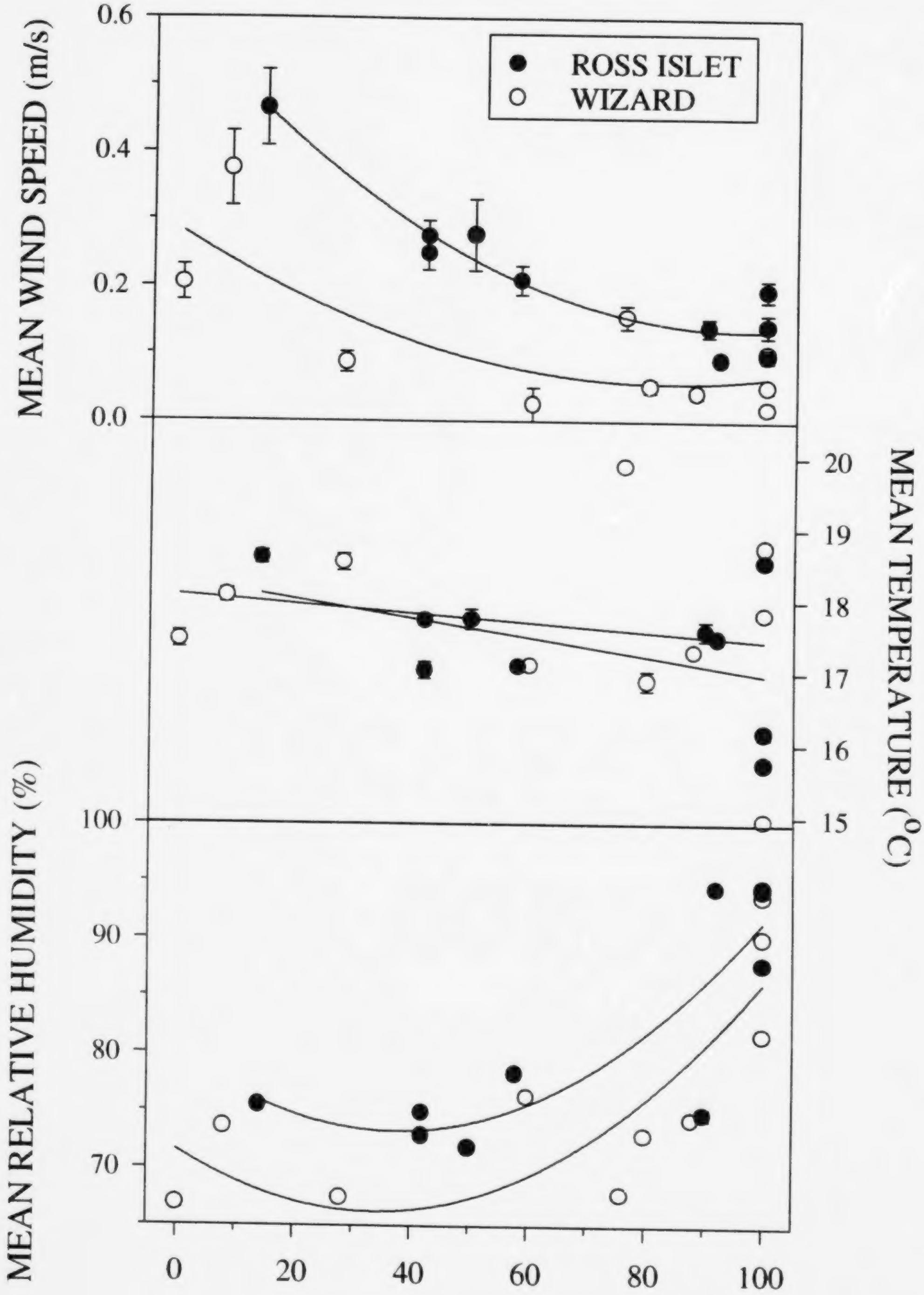

MEAN WIND SPEED (m/s)
0.6
0.4
0.2
0.0
● ROSS ISLET
○ WIZARD
MEAN TEMPERATURE (°C)
20
19
18
17
16
15
MEAN RELATIVE HUMIDITY (%)
100
90
80
70
0
20
40
60
80
100
PERCENT ALGAL COVER

Figure 4-16. Wind velocity, air temperature and relative humidity over 8 h of tidal emersion on a vertical rock face in Grappler Inlet on July 7, 1993. Recordings were made at 20 minute intervals at a height of 10 mm above the substratum. Each temperature and humidity measurement represents the average (± SE) of six measurements taken at 10 second intervals. Each wind velocity measurement represents the average (± SE) of 10 measurements taken at 10 second intervals. Where error bars are not visible they are less than the diameter of the symbol.

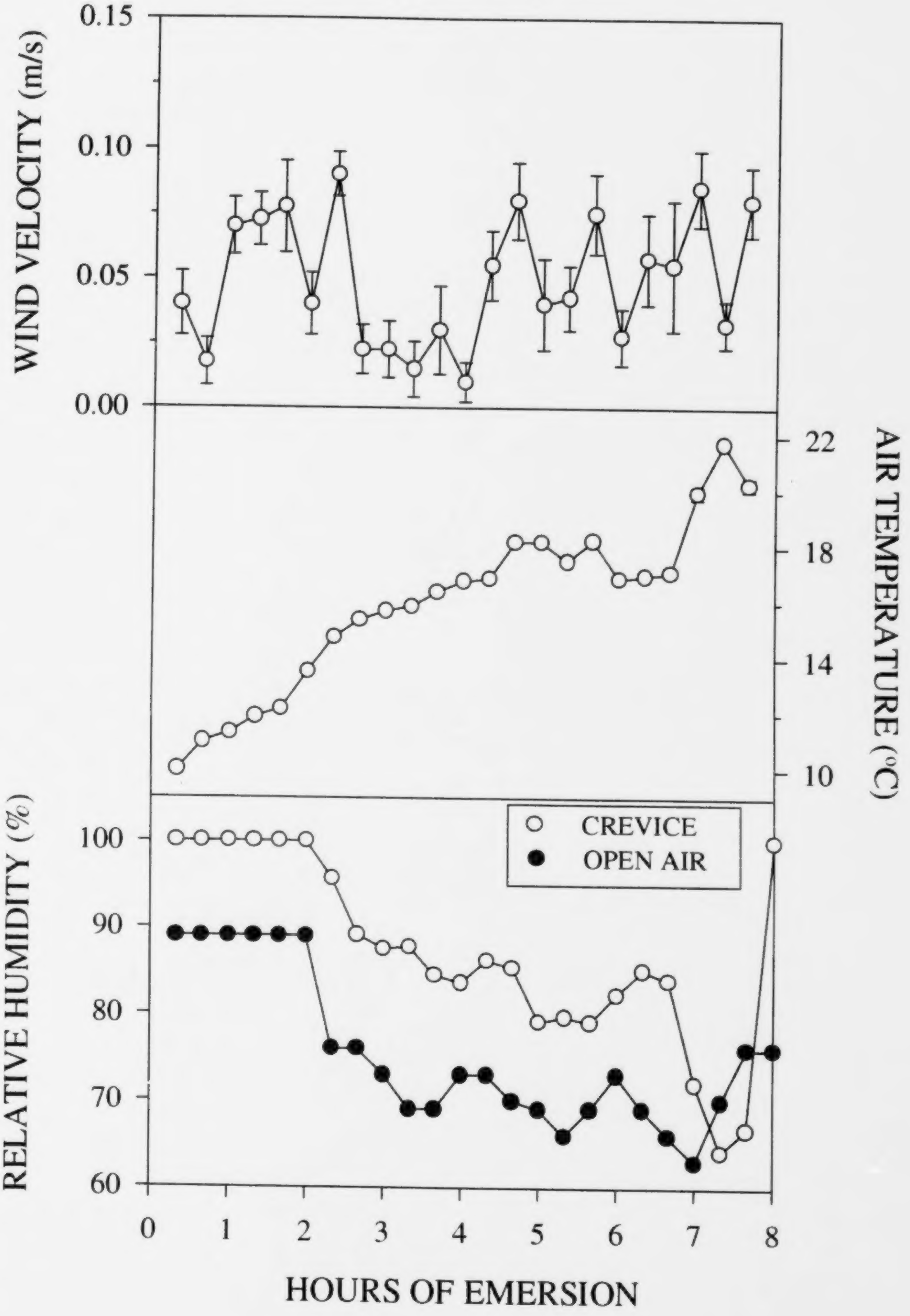

WIND VELOCITY (m/s)
0.15
0.10
0.05
0.00
AIR TEMPERATURE (°C)
22
18
14
10
RELATIVE HUMIDITY (%)
100
90
80
70
60
○ CREVICE
● OPEN AIR
0
1
2
3
4
5
6
7
8
HOURS OF EMERSION

Figure 4-17. Measurements of wind velocity, air temperature, and relative humidity over the course of > 7 h of tidal emersion on a vertical rock face in Grappler Inlet on July 9, 1993. Recordings were made at 20 minute intervals at a height of 10 mm above the substratum. Each temperature and humidity measurement represents the average (± SE) of six measurements taken at 10 second intervals, whereas wind velocity measurements represent the average (± SE) of 10 measurements taken at 10 second intervals. Where error bars are not visible they are less than the diameter of the symbol.

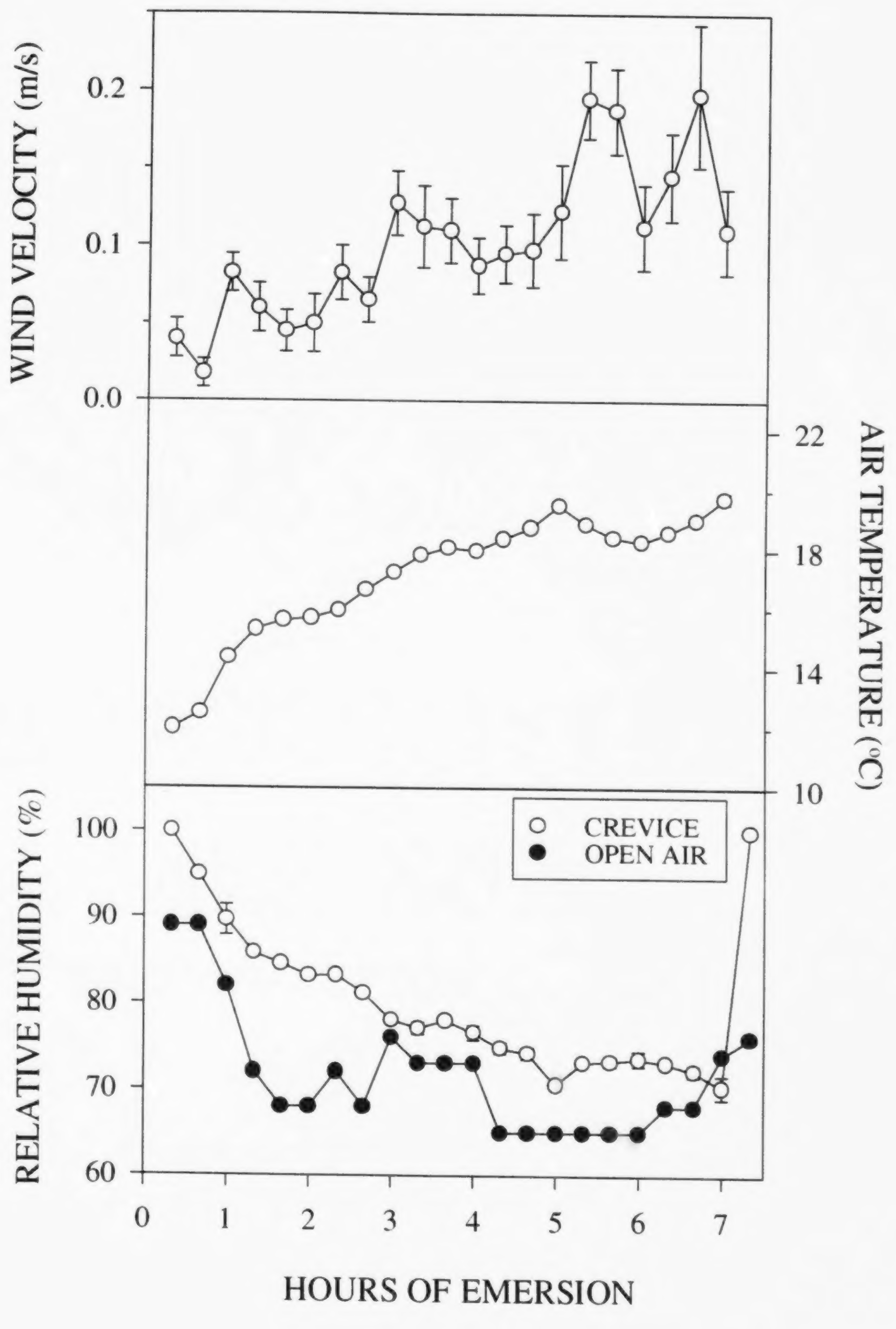

WIND VELOCITY (m/s)
0.0
0.1
0.2
AIR TEMPERATURE (°C)
10
14
18
22
RELATIVE HUMIDITY (%)
60
70
80
90
100
○ CREVICE
● OPEN AIR
0
1
2
3
4
5
6
7
HOURS OF EMERSION

Figure 4-18. Mean percentage (± SE) of the capsule volume lost for "isolated" and "aggregated" capsules of *N. emarginata* when exposed to 7 - 8 h of tidal emersion in Grappler Inlet on July 7, and July 9, 1993. Six isolated and six aggregated capsules were paired together in one of four separate locations along a transect line. Environmental conditions experienced during this time are given in Figures 4-16 and 4-17. Control capsules (n = 12) refer to capsules exposed to air during transport of experimental capsules back to the laboratory. A two-way ANOVA was used to compare the % loss of water (arsine transformed) from isolated versus aggregated capsules, and among locations along the transect line, for each date examined. For July 7, capsule grouping, but not location, had a significant effect on the loss of water from egg capsules (F(grouping) = 10.73; df = 1, 3; $P = 0.05$; F(location) = 1.34; df = 1, 40; $P = 0.27$; F(interaction) = 0.82; df = 3, 40; $P = 0.49$). For July 9, location had a significant effect, but the effect of capsule grouping was only marginally significant (F(location) = 4.08; df = 3, 40; $P = 0.01$; F(grouping) = 6.42; df = 1, 3; $0.05 < P < 0.1$; F(interaction) = 1.83; df = 3, 40; $P = 0.16$).

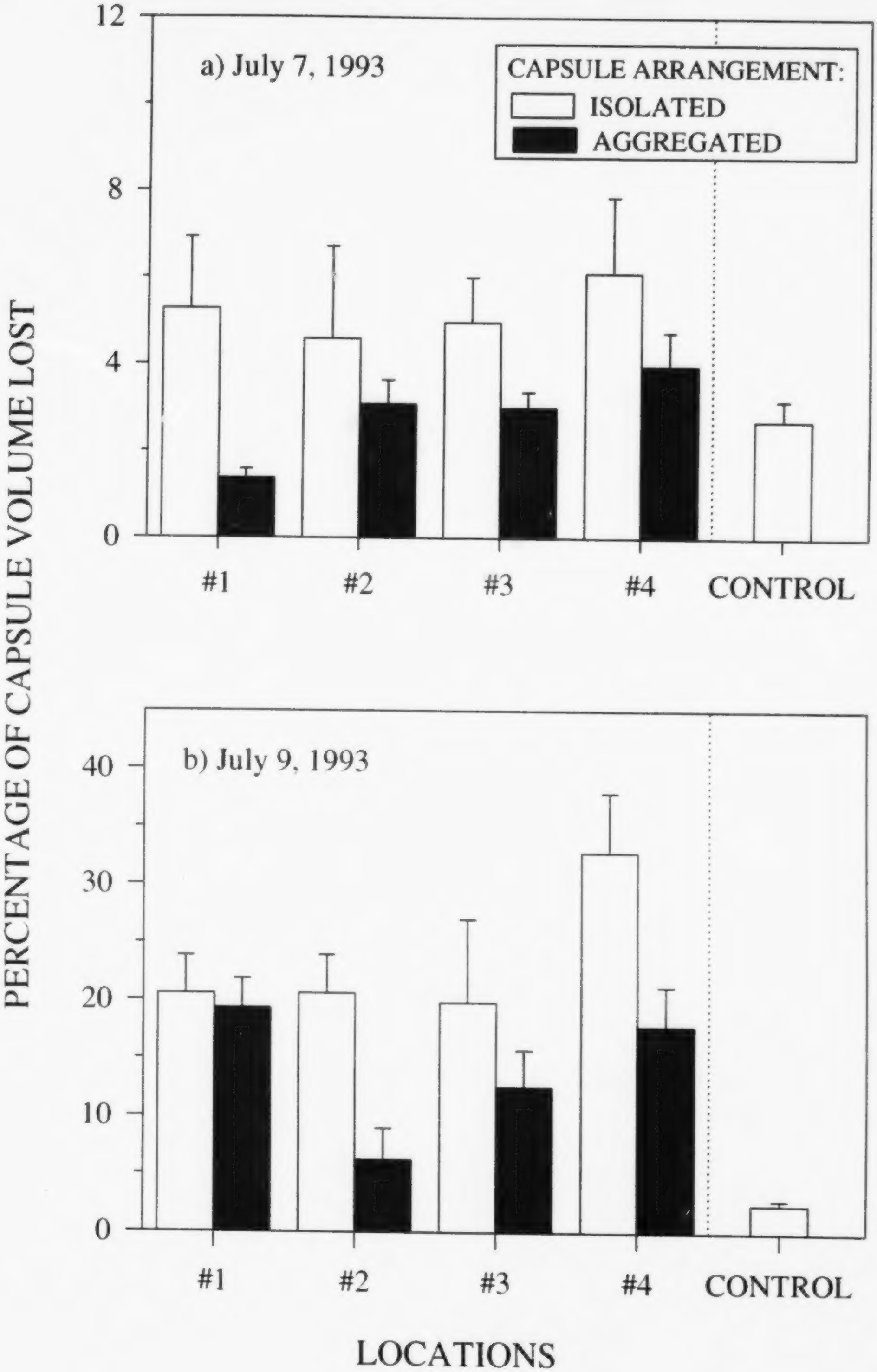

a) July 7, 1993
CAPSULE ARRANGEMENT:
ISOLATED
AGGREGATED
12
8
4
0
#1
#2
#3
#4
CONTROL
b) July 9, 1993
40
30
20
10
0
#1
#2
#3
#4
CONTROL
PERCENTAGE OF CAPSULE VOLUME LOST
LOCATIONS

Figure 4-19. Measurements of wind velocity, air temperature, and relative humidity over the course of 8 h of tidal emersion on a westerly-facing sloping rock face at Ross Islet on Sept 12, 1993. Recordings were made at 20 minute intervals within a quadrat with 52% algal cover at a height of 10 mm above the substratum. Each temperature and humidity measurement represents the average (± SE) of six measurements taken at 10 second intervals. Wind velocity measurements represent the average (± SE) of 10 measurements taken at 10 second intervals. Where error bars are not visible they are less than the diameter of the symbol.

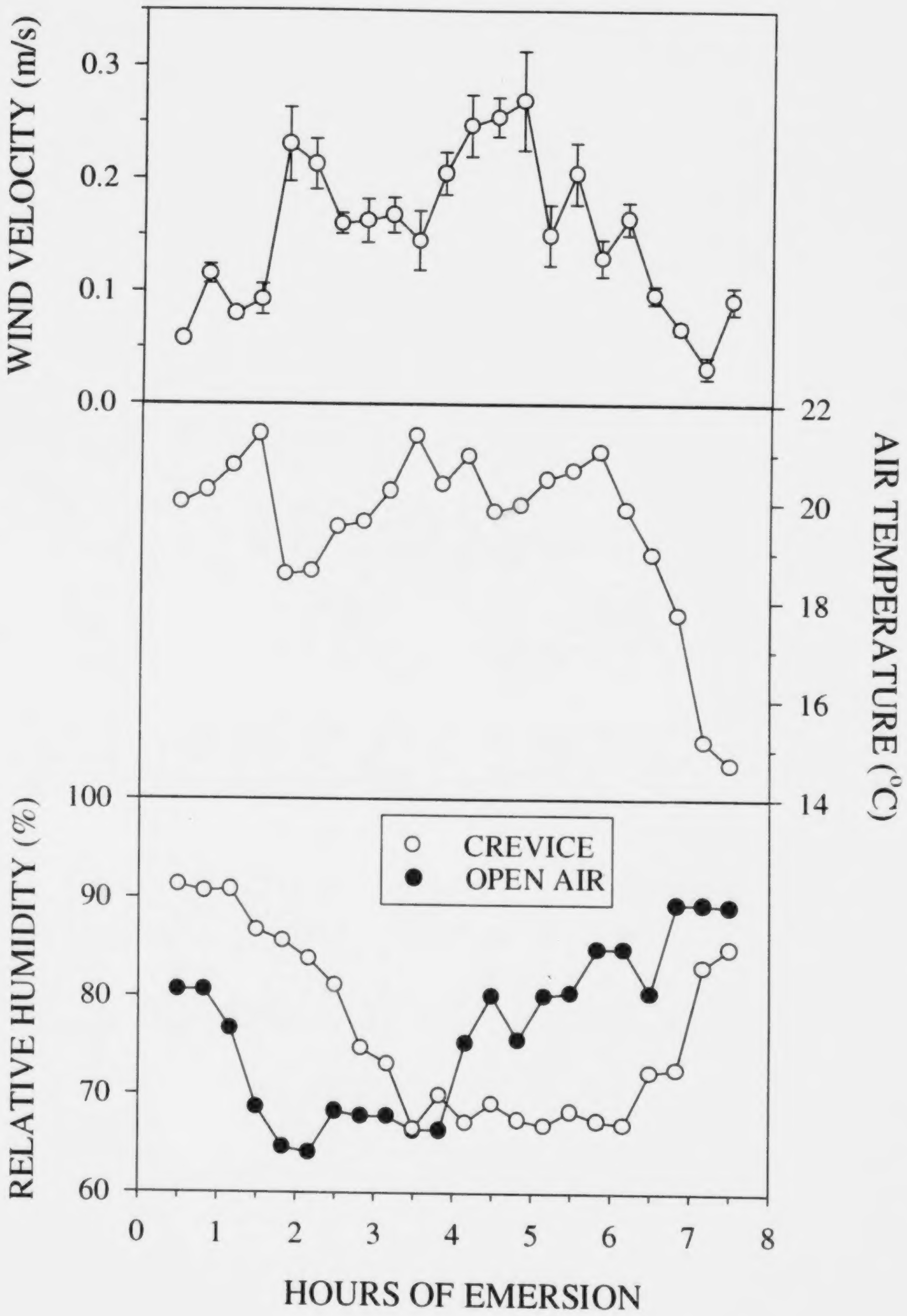

WIND VELOCITY (m/s)
0.3
0.2
0.1
0.0
AIR TEMPERATURE (°C)
22
20
18
16
14
RELATIVE HUMIDITY (%)
100
90
80
70
60
○ CREVICE
● OPEN AIR
0
1
2
3
4
5
6
7
8
HOURS OF EMERSION

Figure 4-20. Mean percentage (± SE) of the capsule volume lost for "isolated" and "aggregated" capsules of *N. emarginata* when exposed to 7 - 8 h of tidal emersion at Ross Islet on Sept. 12, 1993. Microclimatic conditions during this period of exposure are given in Figure 19. Six isolated and six aggregated capsules were paired together in one of five separate locations along a transect line that differed in the extent of algal cover. Control capsules (n = 12) refer to capsules that were exposed to air only during transport of experimental capsules back to the laboratory.

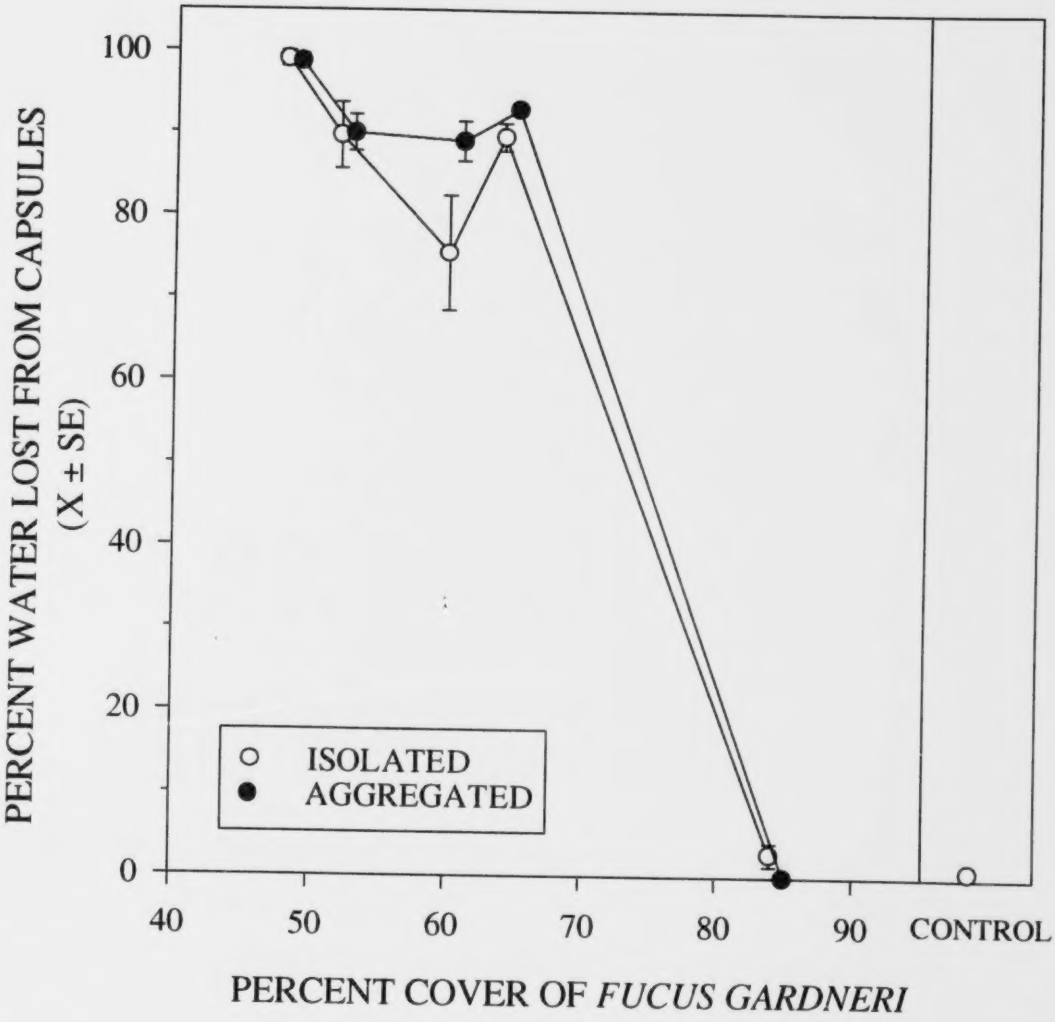


Figure 4-21. The percentage of water lost from thick- (79.1 μm) and thin-walled (52.6 μm) cylindrical egg capsules (6 mm x 3 mm) when modeled under natural field conditions recorded at Grappler Inlet and Ross Islet in 1993. Microclimatic conditions on these days are shown in Figs. 4-16, 4-17 and 4-19. The dotted horizontal line represents the approximate amount of water loss required to kill all embryos within these capsules (i.e., 80%).

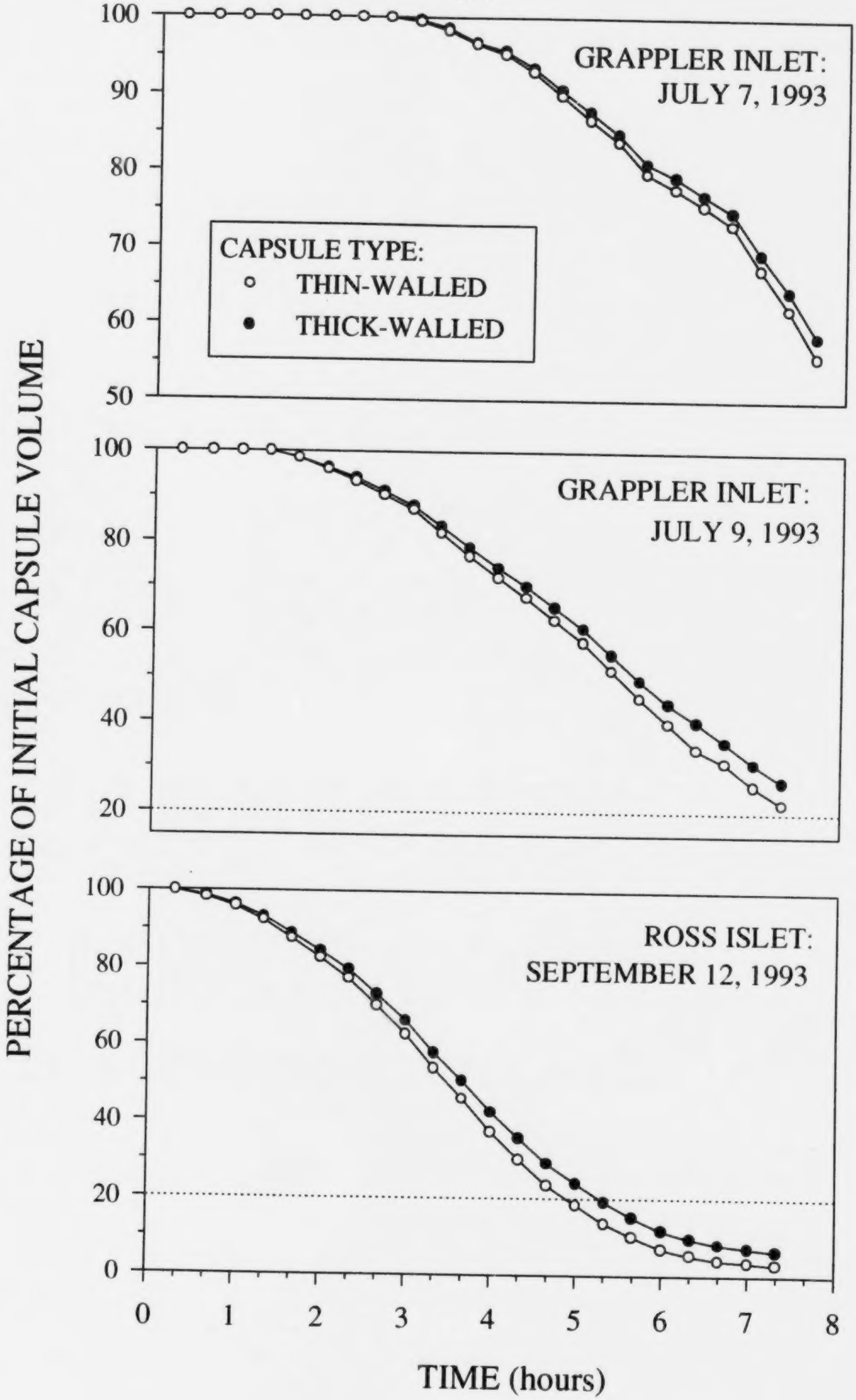
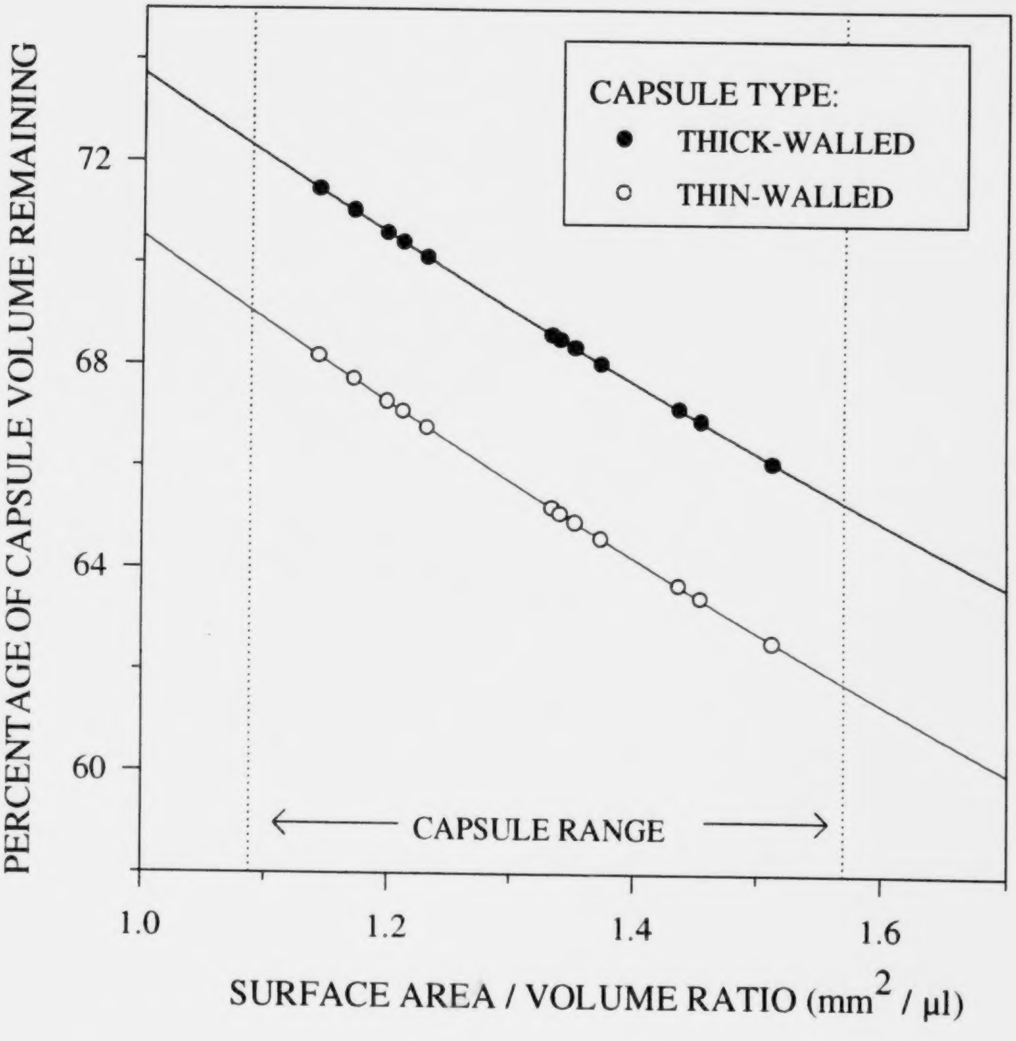

GRAPPLER INLET:
JULY 7, 1993
CAPSULE TYPE:
○ THIN-WALLED
● THICK-WALLED
GRAPPLER INLET:
JULY 9, 1993
ROSS ISLET:
SEPTEMBER 12, 1993
PERCENTAGE OF INITIAL CAPSULE VOLUME
TIME (hours)
100
90
80
70
60
50
100
80
60
40
20
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8

Figure 4-22. The percentage of the capsule chamber volume remaining for cylindrical capsules of differing surface area / volume ratios, and two different wall thicknesses (thick: 79.1 µm, and thin: 52.6 µm) when modeled for 3 h exposure to microclimatic conditions at Ross Islet on Sept. 12, 1993. Regression equations for each line are: thick-walled capsules: $Y = 2.517 X^2 - 21.261 X + 92.480$; thin-walled capsules: $Y = 2.682 X^2 - 22.334 X + 90.202$. The two vertical dotted lines represent the two extremes in shape for field-collected *N. emarginata* capsules from Grappler Inlet (low SA/VOL ratio extreme) and Ross Islet (high SA/VOL ratio extreme) when modeled as cylinders.



CHAPTER 5

Shields Against Ultraviolet Radiation? An Additional Protective Role for the Egg Capsules of Benthic Marine Gastropods.

Abstract

The encapsulation of developing eggs within structurally-complex benthic egg capsules or gelatinous egg ribbons is generally thought to protect developing embryos from environmental risks associated with a benthic existence, such as predation, bacterial attack, desiccation, and osmotic shock. Surprisingly, ultraviolet radiation (UV) has rarely been considered to be a potential source of mortality for encapsulated gastropod embryos, even though natural levels of UV may have profound effects on other marine organisms.

In the present study, I examined the exposure of benthic egg capsules of the rocky shore marine gastropod, *Nucella emarginata*, to direct solar radiation under natural field conditions, and assessed the degree to which capsule walls may protect developing embryos from UV. Although egg capsules of *N. emarginata* were laid under dense algal canopies at some intertidal sites, at more wave-exposed locations egg capsules were deposited in areas directly exposed to solar radiation. Developing embryos within artificially stripped egg capsules were sensitive to and killed by natural levels of UV when exposed to direct solar radiation. In whole egg capsules, however, the multi-laminated wall of *Nucella* capsules provided embryos with substantial protection from the potentially lethal effects of UV.

Capsule walls of *Nucella emarginata* transmitted, on average, < 5% of incident UV-B radiation and < 55% of incident UV-A radiation. Significant differences were evident in the spectral properties of *N. emarginata* capsules collected from different intertidal locations and also among capsules laid by different species of *Nucella*. This variation was associated with intra- and interspecific differences in the thickness of capsule walls, suggesting that UV-absorption is largely a function of the thickness of the barrier separating embryos from their external environment. The actual mechanism by which capsules shield embryos from UV-B radiation remains unclear. No evidence was found for the presence of methanol-soluble UV-absorbing compounds in the walls of *Nucella* capsules, even those these compounds are common in many benthic marine invertebrates. UV absorption, however, may be associated with the presence of fluorescent chromophores involved in the extensive cross-linking of capsule wall proteins. Whatever

the case, these results suggest that the walls of *Nucella* capsules may play an important role in protecting developing embryos from the potentially lethal effects of UV radiation.

Introduction

During the past ten years, there has been considerable interest directed towards understanding the impact of natural levels of solar ultraviolet radiation (UV; 200 - 400 nm) upon marine organisms, and the ramifications of these effects upon whole marine communities. With the recent discovery of an upward trend in the amount of UV reaching the earth's surface (e.g., Smith et al., 1992; Kerr and McElroy, 1993) and the realization that UV can penetrate deeper into the water column than previously believed (Smith et al., 1992; Herndl et al., 1993), these studies have focused on 1) the negative effects of UV on various natural populations of aquatic organisms (e.g., Damkaer and Dey, 1983), 2) the mechanisms by which organisms protect themselves from short wavelength radiation (e.g., Dunlap et al., 1986; Karentz et al., 1991a; Shick et al., 1992; Stochaj et al., 1994), and 3) the implications that increased levels of UV may have for intertidal and shallow water communities (Jokiel, 1980; Herndl et al., 1993).

Natural levels of ultraviolet radiation can have a variety of negative effects on marine organisms. These effects have been attributed to wavelengths of light in the UV-B (280 - 320 nm) and the UV-A region (320 - 400 nm) of the ultraviolet spectrum, since wavelengths below 286 nm cannot penetrate the earth's atmosphere. Although UV-B radiation is believed to cause the most biological damage per unit of energy relative to UV-A (e.g., Behrenfeld et al., 1993), natural levels of both UV-B and UV-A can be genetically, physiologically, and photosynthetically detrimental to many aquatic organisms (e.g., Calkins and Thordardottir, 1980; Jokiel, 1980; Smith et al., 1992; Behrenfeld et al., 1993; Herndl et al., 1993; Blaustein et al., 1994, and references therein). Laboratory and field studies of shallow water plankton communities, for instance, have documented effects of natural exposure to UV such as: a) the suppression of bacterioplankton activity in the top 5 m of near-shore oceanic waters (Herndl et al., 1993), and b) the inhibition of growth in marine phytoplankton communities (Karentz et al., 1991b; Smith et al., 1992). Other studies of benthic organisms have also implicated UV in the necrosis and death of marine epifaunal organisms (Jokiel, 1980) and corals (Gleason and Wellington, 1993). Hence, natural levels of UV can have substantial impacts on marine communities and may also play an important role in limiting the distribution of marine organisms.

Many shallow-water marine organisms have developed mechanisms to reduce their exposure to UV or limit the effects of prolonged exposure to solar radiation. Some species, for instance, specifically avoid habitats associated with high intensities of solar radiation and may suffer substantially lower growth rates or mortality when transplanted to or confined in areas with higher UV irradiance (Jokiel, 1980; Jokiel and York, 1982; Pennington and Emlet, 1986; Gleason and Wellington, 1993). Other aquatic organisms exhibit an increased tolerance to UV (see Calkins and Thordardottir, 1980), that is associated with: a) higher levels of enzymes involved in the repair of nucleic acids damaged by UV (Karentz et al., 1991b; Blaustein et al., 1994), b) increased pigmentation (Garcia-Pichel and Castenholz, 1991), or c) the presence of UV-absorbing or reflecting compounds in the body (Shibata, 1969; Cheng et al., 1977; Jokiel and York, 1982; Dunlap et al., 1986; Karentz et al., 1991a; Shick et al., 1992; Stochaj et al., 1994). In fact, many organisms may depend on one class of UV-absorbing compounds known as mycosporine-like amino acids (MAAs), with absorption maxima between 310 - 360 nm, for increased protection from harmful ultraviolet rays. One recent survey of Antarctic marine organisms, for instance, found detectable levels of MAAs present in macroalgae, sponges, cnidarians, flatworms, nemerteans, polychaetes, molluscs, crustaceans, byozoans, echinoderms, and chordates (Karentz et al., 1991a). Higher concentrations of MAAs have also been recorded in shallow versus deeper water populations of corals (Dunlap et al., 1986) and limpets (Karentz et al., 1992), and in locations of the body more vulnerable to exposure to UV (Shick et al., 1992), thus suggesting a definite association between exposure to solar radiation and defenses against UV. The presence of such behavioral responses and chemical defenses against UV, suggests that natural levels are biologically significant in aquatic ecosystems.

Although considerable attention has been directed towards understanding the effects of UV on adult stages of shallow water benthic marine invertebrates (primarily corals), substantially less is known about the susceptibility of their larvae to UV. Alternate stages in the life-history of many marine organisms may experience extremely different light regimes (see Thorson, 1964). For example, approximately 70% of benthic marine species produce free-swimming planktotrophic larvae (Thorson, 1950). With limited powers of locomotion and at the mercy of water currents for dispersal, these larvae may be exposed to considerably higher doses of UV during development than benthic adults confined to shaded or deeper water habitats. Direct exposure of developing embryos/larvae to artificial UV sources in the laboratory has resulted in such stage-specific effects as abnormally delayed cleavage (Giese, 1938, as cited in Worrest, 1982), photokinetic responses (Pennington and Emlet, 1986; and references therein), or death (Damkaer and

Dey, 1982; 1983; Pennington and Emlet, 1986). The large buoyant eggs and later staged larvae of some echinoderm species may be partially protected from the potentially mutagenic effects of UV by strong pigmentation (Pennington and Emlet, 1986; Griffiths, 1965, Ryberg 1980, as cited in Pennington and Emlet, 1986). Likewise, other larvae may survive periods of intense UV irradiance by diel migrations into deeper water (Pennington and Emlet, 1986) or through biochemical protection associated with the allocation of high concentrations of MAAs to egg cells (Karentz, 1994). Further research is necessary, however, to determine how common these responses are among the larvae of benthic marine invertebrates.

Many benthic marine invertebrates have evolved means of protecting their embryos from environmental risks associated with a planktonic existence by packaging them within egg cases directly attached to benthic substrata. The encapsulation of eggs with these protective coverings is a common phenomenon among such diverse invertebrate phyla as the Platyhelminthes, Nemertinea, Annelida, and Mollusca. Within these groups, eggs can be deposited within a variety of structures, ranging from tough multi-laminated egg capsules to soft gelatinous egg ribbons and jelly masses. Although a number of studies have examined the ability of these structures to protect developing embryos from attack by bacteria and protists (Lord, 1986; Chapter 6), desiccation (Pechenik, 1978; Chapter 4), predation (e.g., Spight, 1977; Brenchley, 1982; Rawlings, 1990; 1994; Chapter 3), and osmotic stress (Pechenik, 1982; 1983), little is known of the role that benthic egg capsules and gelatinous egg masses may play in protecting developing embryos from the potentially lethal effects of UV (but see Biermann et al., 1992).

In this study I provide the first examination of the spectral properties of the egg capsules of a marine gastropod. The marine snail, *Nucella emarginata,* is a common inhabitant of the rocky intertidal zone from Alaska to California and ranges across wide extremes in wave-exposure. It deposits eggs year-round within 6-10 mm-long vase-shaped egg capsules, which are attached to firm substrata in the intertidal zone. Egg capsules are structurally complex and composed of both a thin inner wall and a thick outer capsule wall (Rawlings, 1990; 1995). Each capsule can contain up to 55 embryos (Rawlings, unpub. data) which spend 2-3 months developing within the capsule before hatching out as juvenile snails (see Strathmann, 1987). The objectives of this study were threefold: 1) to assess the relative exposure of naturally deposited egg capsules of *Nucella emarginata* to solar radiation at three intertidal sites in Barkley Sound on the west coast of Vancouver Island, 2) to examine the spectral properties of capsular cases of *N. emarginata* and two other local intertidal species of *Nucella*, and 3) to determine the vulnerability of encapsulated embryos to natural levels of ultraviolet radiation.

Materials and Methods

Survey of naturally-deposited egg capsules of *Nucella emarginata*

In August 1994, I conducted a field survey to document the range of intertidal microhabitats used as spawning sites by *Nucella emarginata*, and to assess the potential exposure of naturally-deposited egg capsules to direct solar radiation within these microhabitats. Three intertidal sites were chosen for this survey; the choice of these specific locations was based on their exposure to wave action, and also on the local availability of intact egg capsules at these sites during the period this survey was conducted. These sites were: 1) Ross Islet (48°52'12" N, 125°9'42" W), 2) Wizard Rock (48°51'24" N, 125°09'36" W), and 3) Kirby Point (48°50'42" N, 125°12'24" W). These have been ranked in wave exposure based on 1) the maximum height of the *Balanus glandula* zone, 2) the lowest height of vascular plants, and 3) offshore measures of wave height; all three measures have placed these sites in the following order along a gradient of increasing wave-exposure: Ross Islet < Wizard Rock < Kirby Point (Palmer et al., unpub. data).

At each site, a 10 m stretch of shoreline was selected in a region where *Nucella emarginata* and their egg capsules were known to be abundant. A 5-10 m long transect line was then placed parallel to the waterline, at a tidal height that intersected the vertical distribution of *N. emarginata* (Ross Islet: 2.43 m [above ELWS, chart datum]; Wizard Rock: 2.91 m; Kirby Point: 3.20 m). To determine the local availability of microhabitats for capsule deposition within the selected intertidal area, I placed a 0.1 x 0.1 m quadrat (divided into 25 x 0.004m^2 grids) at intervals of either 0.25 or 0.5m along the transect line. Within each quadrat, I estimated the percent cover of any algal canopy (e.g., *Fucus gardneri, Mastocarpus papillatus*) covering the primary substrate. This algal cover was then removed and the percent cover of all understorey microhabitats estimated.

To determine the microhabitats specifically used for capsule deposition by *Nucella emarginata*, I searched for 20 groups of intact egg capsules within 0.25m of the transect line. Once a group of capsules was found, it was marked by attaching a piece of fluorescent flagging tape next to the capsule mass. Because any algal cover overlying these capsules had to be displaced to locate and mark each group of capsules, I could not estimate the relative exposure of these capsules to direct solar radiation on the same day that they were tagged. Upon returning to these sites, usually on the following day, I noted whether or not capsules were visible prior to displacing any of the algal canopy. If capsules were visible, I measured the vertical and horizontal angle of unobstructed exposure to sunlight using a modified map divider and protractor (see Fig. 5-1), and also the net direction of exposure using a compass. Once these measurements were made, the quadrat was centered over the group of capsules, and the percent cover of algal canopy and understorey microhabitats was estimated, as described above.

The specific substrata on which capsules were deposited was also recorded for each group of capsules. Because initial observations indicated that capsules were laid almost exclusively on bare rock and the shells of the barnacle, *Semibalanus cariosus,* I categorized capsules according to whether or not they were laid 1) on bare rock, nestled against other organisms, 2) inside *S. cariosus* tests, 3) on the outside surfaces of *S. cariosus* tests, or 4) on open bare rock. This classification was intended to provide a crude index of the relative degree of exposure of egg capsules to desiccation and solar radiation (1 = most protected, 4 = most exposed); this ranking did not take into consideration the potential ameliorating effects of an overlying algal canopy, if present. This procedure was repeated for all three study sites.

In early September 1994, I examined a fourth intertidal site located inside the entrance to Execution Cave (48°48'48" N, 125°10'36" W). This site was of interest because of the unusual conditions associated with the cave environment (high humidity, low solar radiation) and the marked difference between microhabitats that were selected for capsule deposition at this site compared to other intertidal locales. I quantified this difference by placing 0.1 x 0.1 m quadrats over the first 20 groups of capsules found within the cave, and recorded the percent cover of microhabitats around each group of capsules and also the specific substrata on which capsules were deposited.

Spectral properties of *Nucella* egg capsules

The spectral properties of *Nucella* egg capsules were measured using a fiber optic probe system designed by Vogelmann and Björn (1984). This system was built to examine light absorption through thin layers of plant leaves and fir needles (Vogelmann and Björn, 1984; Vogelmann et al., 1988; Vogelmann et al., 1991), but was easily adapted to working on the inert capsule walls of *Nucella* species. To examine light transmittance through capsule walls, capsules were opened, emptied of contents, and rinsed with fresh water. A small piece of capsule wall (approx. 1.5 x 1.5 mm) was then cut from the middle region of the capsule case and mounted between two plastic coverslips. Each coverslip had a 1 mm^2 hole drilled at identical positions, such that when these two holes were superimposed light could travel across the exposed capsule wall without being filtered by the plastic coverslips. A piece of wet absorbent tissue was also compressed between the plastic coverslips to ensure that the capsule piece remained moist throughout each trial. Once the capsule was secure, the coverslips were mounted in a permanent holder positioned a fixed distance (180 mm) from a light source. Collimated light, provided by a 150 W xenon arc lamp, was focused on the outer surface of the capsule piece. The light gathering end of a modified fiber optic probe (16 μm in diameter), mounted in the eye of a needle and attached to a micromanipulator, was then brought to within 0.06 mm of the inside layer of the capsule wall. The opposite end of the fiber optic probe was connected to a spectroradiometer interfaced with an IBM computer. All data were recorded using custom-written software.

Once the capsule piece was mounted and positioned in the light path, I recorded the intensity of radiant energy crossing the capsule piece at 2 μm intervals for a range of wavelengths spanning both the UV-A (320 - 380 nm, including one measurement at 400 nm) and the UV-B region (280 - 380 nm). Although the percent transmittance of light through pieces of capsule wall varied slightly among samples from different regions of the capsule, these differences were not significant (data not shown). Hence, samples taken from the middle region of the capsule wall were chosen to be representative of the whole capsule. Each experimental trial was replicated three times using the same capsule piece. For each "within capsule" replicate, the fiber optic probe was kept at the same distance from the light source, but was moved to a new position ≈ 0.2 mm on either side of the original measurement. Each experimental trial was calibrated against a control trial (3 replicates) in which the fiber optic probe was positioned at the same distance from the xenon light source, with plastic coverslips and stand in place, but without a capsule piece present. Experimental trials and their respective control trials were always run consecutively to minimize any error associated with a temporal change in the output from the xenon light source. Percent transmittance values for capsule pieces were calculated by dividing the light transmittance values for experimental trials by the average transmittance for each control trial. A correction was applied to all measurements of light transmittance to negate the effect of light scattering by capsule walls (see Day et al., 1992). To do this, recordings of light transmittance were made at 500 nm for a representative group of all capsules used in this study. Based on the color of these capsules, at this wavelength relatively little light should be absorbed by the capsule wall, such that any reduction in light intensity due to the capsule piece must result from light scattering. Thus, transmittance values in the UV-A and UV-B range were corrected by dividing by the percentage of light transmitted across the capsule wall at 500 nm.

A slight modification of the above technique allowed measurements of light transmittance to be made at various depths within the capsule wall. To examine this, the fiber optic probe was advanced until it just made contact within the inner surface of the outer capsule wall; the orientation and position of the probe were guided with the use of a Gaertner microscope. The fiber optic probe was then driven through the capsule wall by a stepping motor (0.5 - 1.0 µm steps). Light readings were recorded at 2 µm intervals through the capsule wall for a representative wavelength of 300 nm. The percentage of light transmitted through the capsule wall was determined by dividing the amount of light detected at specific depths in the capsule wall by the amount of light detected once the probe had completely penetrated the capsule piece. A correction due to scattering was applied by fitting a curve to the spectral measurements of light transmittance with depth of penetration (in percent) for scans at 300 nm and 500 nm for the same capsule pieces. Once these functions had been determined, transmittance values at 300 nm were divided by the percentage of light transmitted at 500 nm for specific depths through the capsule wall.

Using these techniques, I examined variation in spectral absorption properties of capsule walls collected from three different intertidal populations of *Nucella emarginata*: Grappler Inlet (48°49'54" N, 125°06' 54"W), Ross Islet, and Kirby Point. Capsules were collected in early May 1994, by searching for freshly deposited capsules and removing one capsule from each group. Capsules were also collected from a laboratory population of *N. emarginata*, consisting of snails born and raised entirely within the laboratory. Because preliminary results indicated that *Nucella* capsule walls did absorb a substantial amount of UV-B radiation, initially I examined which portion of the capsule wall was chiefly responsible for absorbing UV. To do this, I separated the inner and outer capsule wall from several capsules (see Rawlings, 1995, or Chapter 6, for a photograph of these two regions of the capsule wall), and tested these different portions of the capsule wall separately. All subsequent comparisons of UV transmittance among populations of *N. emarginata* examined the filtering capacity of both capsule portions together (except for measurements taken at distances through the capsule wall, in which only the outer wall was used).

I also tried to determine if any UV-absorbing compounds could be extracted from the walls of *Nucella emarginata*. Previous studies have found 80% methanol to be a good solvent for extracting mycosporine-like amino acids involved in UV-absorption in marine organisms (Karentz et al., 1991a). To conduct this experiment, 5 capsules of *N. emarginata* were bisected and rinsed in sterile seawater to remove their contents. One half of each capsular case was then placed in 5 ml of sterile seawater, while the matching piece was deposited in 5 ml of 80% methanol. All capsules pieces were kept at 4°C for 24 hr; previous studies have shown that MAAs are extracted by methanol over the course of only a few hours (e.g., Karentz et al., 1991a). After this period, the spectral properties of each capsule piece were compared across UV-A and UV-B ranges to determine if there was any change in light transmittance associated with the extraction of UV-absorbing compounds from the methanol-treated capsule pieces.

The spectral properties of *N. emarginata* capsule walls (from Kirby Point) were also compared to the spectral properties of *Nucella lamellosa, and N. canaliculata* egg capsules. Egg capsules of *Nucella lamellosa* and *N. canaliculata* were collected from Grappler Inlet and Kirby Point, respectively, in early May, 1994. Methods used to examine the spectral properties of these capsules were identical to those described above for *N. emarginata* capsules.

Vulnerability of encapsulated embryos to ultraviolet radiation

From July to September 1993, I conducted two sequential outdoor experiments to examine the relative vulnerability of embryos within stripped and whole capsules to ultraviolet radiation. Both experiments followed the same protocol. For Experiment I, paired capsules were collected from a laboratory population of *Nucella emarginata*. One capsule from each pair was stripped of two panels of its outer capsule wall, as described previously ("stripped", Rawlings, 1995; Chapter 6), and the other was left intact (hereafter termed "whole"). All capsules used in this experiment contained late third-stage or early fourth-stage veliger embryos, as defined by LeBoeuf, (1971). Paired capsules were arranged side by side on one ray of a six-rayed Tygon™ holder (Rawlings, 1995; Chapter 6). In total, six capsule pairs were positioned on each holder. Once 12 holders had been filled with capsules, each holder was placed in a sterilized culture jar containing 250 mls of autoclaved, antibiotic-treated (0.050 g/l penicillin; 0.030 g/l streptomycin) seawater, such that capsules were suspended approximately 20 mm below the surface of the water. Each jar was then randomly assigned to one of three sunlight treatments: 1) no sunlight, 2) sunlight filtered to remove UV-A and UV-B radiation (UV-absorbing), and 3) sunlight filtered to remove wavelengths shorter than UV-A and UV-B (UV-transmitting). These treatment conditions were created by mounting either an opaque Plexiglas cover, or a UV-transmitting or UV-absorbing Acrylite cover on the top of each culture jar. The spectral properties of each Acrylite plate were confirmed using a UV visible Perkin-Elmer Spectrophotometer (Coleman 139) (Fig. 5-2).

Once assigned to treatment conditions, culture jars were inserted into a rack (Fig. 5-3) mounted in an outdoor seawater tank. The rack consisted of a wooden frame with four removable opaque plastic trays. Each tray had three wells into which a culture jar could be positioned. Treatment conditions were arranged in a replicate block design (see Fig. 5-

3), such that one jar from each treatment was placed in the same tray. Because the jars sat snugly within their wells, the only light that entered each jar had to pass through the Acrylite or Plexiglas cover mounted above. Underneath the rack, each jar was bathed in a pool of continuously flowing seawater, thus keeping the water temperature within each jar relatively stable during the course of the experiment (11-13°C). Temperature readings were taken within each jar at irregular intervals to determine if there were any differences among treatments based on the amount of sunlight penetrating each jar. Initial observations also revealed that on sunny days the seawater table and experimental apparatus received direct sunlight from noon until late in the afternoon.

Experiment I was conducted over a period of 15 days extending from July 27 to August 10. During this period, daily UV-B measurements were provided from the Saturna UV Monitoring Station (48° 44'N; 123° 8'W) on the east coast of Vancouver Island, and daily records of the number of hours of sunshine were collected from the nearest weather station in Tofino. The condition of all capsules was checked daily and any obvious changes noted. Acrylite and Plexiglas covers were wiped clean every two days, and at four - five day intervals, the water within each culture jar was replaced with freshly sterilized, antibiotic-treated seawater. The experiment was terminated on day 15 following the appearance of a purplish color in many of the stripped capsules in the treatment exposed to UV. Once the experiment was terminated, all culture jars were brought back into the laboratory. Capsules were opened individually and the number of live and dead embryos were counted. The presence/absence of protists was also noted.

Experiment II followed the same protocol as Experiment I. Capsules used in Experiment II, however, were collected from a field population of snails at Ross Islet. Embryos within Ross Islet capsules were younger than those in capsules used in the first experiment; in fact, most embryos had not yet reached the third-stage veliger prior to the start of the experiment (see LeBeouf, 1971). Experiment II was conducted over a period of 24 days from August 15 to Sept 7, 1993. UV and sunlight measurements were collected from the Saturna Island UV Monitoring Station and Tofino Weather Station, as described previously.

Statistical procedures

Parametric statistics were used in all analyses except where the assumptions of these tests could not be met. Variances were compared for homogeneity prior to undertaking each analysis; in cases where variances were heterogeneous, the data were transformed (arcsine transformed for percentage data), and retested for homogeneity of variances. If variances were still heterogeneous, a parametric test allowing for comparisons among samples with heterogeneous variances was undertaken (e.g. adjusted t-test, Sokal and Rohlf, 1981) or an equivalent non-parametric test was used.

Results

Survey of naturally-deposited egg capsules of *Nucella emarginata*

The availability of specific microhabitats differed substantially among the three study sites chosen for this survey (Fig. 5-4; hollow bars). The most dramatic difference among these sites was the extent of algal canopy covering the primary substratum. At both Ross Islet and Wizard Rock, substantial algal cover was present (> 60%), consisting mainly of *Fucus gardneri* and to a lesser extent *Mastocarpus papillatus*; this algal canopy was totally absent from the Kirby Point study site. The availability of understorey microhabitats also differed among these three sites, primarily in the amount of unoccupied space (bare rock), turf algae, barnacles and mussels.

Microhabitats associated with *Nucella emarginata* capsules were not a random selection of those available (Fig. 5-4; hollow vs. filled bars); some microhabitats were used significantly more frequently than expected based on their availability. The preferences of snails for specific microhabitats also differed substantially among sites (Table 5-1). At Ross Islet, snails selected areas that had a more extensive algal canopy, but a reduced amount of turf algae, relative to available habitats. At Wizard Rock, areas with more algal cover, bare rock and *Semibalanus cariosus*, but a reduced cover of *Mytilus* spp. and *Balanus glandula,* were used as spawning sites. Snails at Kirby point, where there was no algal canopy present, exhibited a significant preference for microhabitats with a greater cover of gooseneck barnacles (*Pollicipes polymerus*) and turf algae.

Although capsules were laid exclusively on bare rock and the shells of *Semibalanus cariosus* at all three sites, the frequency with which these substrata were used differed significantly among study locations (Fig. 5-5). Under the algal canopy at Ross Islet, the majority of capsules were found on open bare rock surfaces, with < 20% of capsules present in more sheltered habitats. Likewise, under the algal canopy at Wizard Rock, snails deposited their capsules almost exclusively on either bare rock surfaces or the outer surfaces of *S. cariosus* tests. At Kirby Point, there was a marked difference in the selection of substrata used for capsule deposition. Although barnacles and bare rock were each used with relatively equal frequency, the majority of capsules were squeezed next to other organisms or deposited within the protective confines of empty *S. cariosus* tests (see Fig. 5-6). Relatively few capsules were found on open rock surfaces.

Despite the lack of any algal canopy at Execution Cave, *N. emarginata* egg capsules were laid in areas with a very sparse covering of mussels and barnacles (< 20% cover), and large areas of bare rock (Figs. 5-4, 5-5). The majority of these capsules were laid on completely exposed surfaces; fewer than 35% were nestled amongst other organisms.

Sites also differed significantly in the exposure of egg capsules to direct solar radiation (Fig. 5-7). This difference largely reflected the presence and extent of the algal canopy overlying *N. emarginata* egg capsules. At Ross Islet and Wizard Rock, with an algal canopy covering > 90% of quadrats containing capsules, *N. emarginata* capsules were very well protected from direct radiation during emersion. The lack of an algal canopy at Kirby Point, however, left capsules considerably more exposed to direct sunlight. Although snails at this site selected habitats likely to be more protected from desiccation and solar radiation (Fig. 5-5), all capsule masses received some exposure to direct sunlight.

Spectral properties of *Nucella* egg capsules

Capsule walls of *Nucella emarginata* were relatively opaque to UV-B radiation (280 - 320 nm), but considerably more transparent to UV-A radiation (320 - 400 nm) (Fig. 5-8). The thick outer wall of *N. emarginata* capsules was chiefly responsible for the absorption of UV. Comparisons between the spectral properties of the inner (≈ 5 μm thick) and the outer capsule wall (≈ 60 - 80 μm thick) indicated that the innermost capsule wall absorbed significantly less UV-A and UV-B radiation than the thick outer capsule wall. Interestingly, capsules collected from three populations of *Nucella emarginata* differed substantially in their light-absorbing properties. Capsules from Ross Islet transmitted significantly more ultraviolet radiation than capsules collected from either Grappler Inlet or Kirby Point (Fig. 5-9); these differences were marginally significant for UV-A and very significant for UV-B radiation. Significant differences were also evident among the spectral properties of *Nucella emarginata* (from Kirby Point), *N. lamellosa* and *N. canaliculata* capsules. Comparisons of light transmittance at 300 nm and 360 nm wavelengths illustrated that *N. lamellosa* capsules were more transparent to UV radiation than either *N. emarginata* or *N. canaliculata* capsules (Fig. 5-10).

Measurement of UV radiation as a function of depth within the wall of *Nucella emarginata* capsules was made by advancing the fiber optic probe through the outer wall at known increments. These measurements showed that 90% of radiation at 300 nm was absorbed within the outer half of the capsule wall (Fig. 5-11a). Measurements through replicate pieces of *N. emarginata* capsules mounted forwards and backwards also revealed no polarity to the outer wall; light transmittance profiles of capsule walls were almost identical, regardless of orientation. The percent transmittance of UV radiation also varied similarly with depth within the outer walls of representative *N. emarginata*, *N. canaliculata* and *N. lamellosa* capsules (Fig. 5-11b), suggesting that differences in the spectral properties of these capsules may be explained simply by differences in the thickness of their walls.

Attempts to extract UV-absorbing compounds from the capsule wall using 80% methanol proved unsuccessful (Fig. 5-12). The spectral properties of capsular cases that had been soaked in 80% methanol for 24 h did not differ significantly from those that had been soaked in sterile seawater for the same period of time.

Vulnerability of encapsulated embryos to ultraviolet radiation

Experiment I. Over the 15 days of this experiment, there was an average of 5.5 ± 1.51 hours (mean ± SE) of sunshine per day. This included 5 days with more than 8 hours of sunshine, 3 days with 4 - 8 hours of sunshine, and 7 days with less than four hours of direct sunshine (Fig. 5-13). Days with little or no recorded sunshine resulted either from extensive cloud cover or from heavy fog often associated with coastal areas of western Vancouver Island in late summer. Because weather conditions at Bamfield were not likely to be identical to those recorded at Saturna Island, UV-B measurements from Saturna were only used to provide an estimate of the maximum intensity of UV radiation during this experiment. The average midday maximum UV-B reading from Saturna over the experimental period was 156.874 ± 9.9013 mW/m^2 (mean ± SE; n = 14).

Despite the relatively few days with prolonged sunshine in Experiment I, sunlight had a significant effect on embryonic survival (Fig. 5-14; Table 5-2): embryos exposed to UV radiation suffered substantially higher mortality than those in the dark or in UV-filtered sunlight treatments. Sunlight, however, had a differential effect on embryonic survival depending or whether or not capsules were whole or stripped. Embryos in stripped capsules experienced significantly higher mortality in the UV-exposed sunlight treatment relative to embryos within whole capsules. Interestingly, of 129 embryos within stripped capsules that died during exposure to UV radiation, 78.3% exhibited a strong purple coloration. This was usually associated with the production of large quantities of mucus near the foot region and is believed to result from secretions of the hypobranchial gland following exposure to physiological stress (e.g., Spight, 1977; Gallardo, 1979; Pechenik, 1982, 1983; Srilakshmi, 1991). This change in coloration was never seen in embryos exposed to other light treatments, or in embryos within whole capsules.

Among-treatment differences in embryonic mortality were unlikely to have resulted from differences in water temperature. Repeated measurements of water temperature within culture chambers during the mid-late afternoon of warm sunny days did not reveal any substantial differences among light treatment conditions (e.g. Aug 2, 1993: Dark: 12.3 ± 0.14 °C; UV-filtered Sunlight: 12.5 ± 0.20 °C; UV-exposed Sunlight: 12.4 ± 0.24 °C [n = 4 replicate measurements]) .

Experiment II. Capsules experienced considerably more days of sunshine over the 23 days of Experiment II: 12 days of > 8 hours sunshine, 4 days with 4 - 8 hours sunshine, and 7 days with < 4 hours sunshine (Fig. 5-13). Although the average number of hours of sunshine per day was higher for this experiment (mean ± SE; 6.7 ± 0.97 hours), the average midday UV-B maximum over this period was lower than that recorded for Experiment I (128.795 ± 4.2232 mW/m^2, n = 19 [mean ± SE]).

The percentage of embryos surviving exposure to different sunlight treatments was generally lower in Experiment II compared to Experiment I (Fig. 5-14; Table 5-2). One series of replicates had to be terminated because cultures became contaminated with protists, which killed developing embryos within most stripped capsules. No other cultures were contaminated, however. Comparisons of embryonic mortality among treatment conditions revealed a significant effect of sunlight treatment. Embryonic survival was significantly lower in the UV-exposed sunlight treatment compared to the dark and UV-filtered sunlight conditions. Although no significant effect of capsule wall treatment (stripped vs. whole) was evident in this experiment, there was a trend towards the lower survival of embryos enclosed within stripped capsules exposed to UV radiation. A two-way non-parametric ANOVA comparing the survival of embryos within UV-exposed stripped and whole capsules between Experiment I and II, revealed both a significant effect of the capsule wall (stripped vs. whole: $\chi^2 = 3.92$, $P < 0.05$) and a significant difference among experiments (Experiment I vs. II; $\chi^2 = 4.82$, $P < 0.05$).

Discussion

The encapsulation of developing eggs within structurally-complex benthic egg capsules or gelatinous egg ribbons has generally been assumed to protect developing embryos from a variety of environmental risks associated with a benthic existence (see Pechenik, 1986, for a review). Surprisingly, ultraviolet radiation has rarely been considered to be a potential source of mortality for encapsulated gastropod embryos (but see Biermann et al., 1992), even though natural levels of UV may have profound effects on marine organisms (e.g., Karentz et al., 1991a). The results of the present study illustrate not only that egg capsules of *Nucella* spp. can be exposed to appreciable amounts of solar radiation under natural conditions, but also that capsule walls may shield embryos from the potentially lethal effects of UV-B radiation. Thus, these findings suggest an additional protective role for the capsular cases of benthic marine gastropods.

Selection of spawning habitats by *Nucella emarginata*

Many species of neogastropods exhibit a remarkable specificity for certain spawning habitats. Individuals of *Nucella lamellosa*, for instance, will often return to exactly the same intertidal spawning locations year after year (Spight, 1974). In other species, the lack of "suitable" substrata for spawning may substantially limit the reproductive output of a population (Brenchley, 1981). Despite this specificity, however, little is known about the role that biological and physical factors, such as predation, desiccation, heat stress, and ultraviolet radiation, may play in governing the selection of these spawning habitats. Although areas exposed to direct solar radiation may be less favored as spawning sites by marine gastropods (e.g. Emlen, 1966; Gallardo, 1979; Biermann et al., 1992; this study), this may not reflect avoidance of ultraviolet radiation, specifically. Higher wavelength radiation, desiccation and/or heat stress are all associated with increased solar radiation, and hence, could also be selective forces influencing the preference of gastropods for shaded spawning areas.

Physical stresses undoubtedly play a role in influencing the selection of spawning habitats by *Nucella emarginata*. At Execution Cave, where the intensities of environmental stresses such as UV radiation, desiccation, and heat stress were relatively benign compared to other sites, the majority of capsules were laid in areas completely unprotected from these stresses. At other sites, where these stresses were relatively more severe, the selection of spawning sites depended on the presence or absence of an algal canopy. Under field conditions where a thick algal canopy of *Fucus* or *Mastocarpus* was available, snails deposited egg capsules in a variety of habitats within the moist and shaded covering of this canopy. At more wave-exposed locales, which typically lack this extensive algal cover (Menge, 1978), snails laid capsules within barnacle tests or near clumps of *Pollicipes polymerus;* capsules within these habitats remained moist during emersion, but were considerably more open to direct solar radiation (e.g., Fig. 5-6). Because populations of *Nucella emarginata* at these exposed locales spawn primarily in the spring and summer months (Gosselin, 1994; Rawlings, unpub. data), and because these populations persist from year to year, encapsulated embryos of this species appear able to tolerate periods of direct exposure to UV radiation. Thus, exposure to UV appears unimportant in the selection of spawning sites by this species. In contrast, however, desiccation stress is likely a very frequent source of mortality for encapsulated embryos of *Nucella* species (Feare, 1970; Spight, 1977; Rawlings, unpub. data). Although encapsulated embryos can withstand up to 80% water loss from the capsule chamber, capsules can desiccate very quickly under summer field conditions unless protected by an algal canopy (Chapter 4) or other moist habitats. Avoidance of desiccation stress may thus explain the preference of *N. emarginata* for the spawning habitats selected in this study.

Spectral Properties of *Nucella* Egg Capsules

The deposition of *Nucella* egg capsules in habitats directly exposed to solar radiation suggests that either capsule walls are effective barriers to UV or that embryos have the ability to repair UV-induced damage. Although no tests were conducted for UV-repair enzymes, the leathery egg capsules of *Nucella emarginata* were relatively opaque to UV-B radiation. Likewise, the capsule walls of *Nucella lamellosa* and *N. canaliculata* also prevented significant amounts of UV from entering the egg capsule. These findings are somewhat surprising given that the encapsulating structures of other organisms appear to provide little protection from UV. The survival of amphibian embryos exposed to natural intensities of solar radiation, for instance, appears to be dependent on the concentration of photoreactive enzymes involved in the repair of UV-damaged DNA (Blaustein et al., 1994), presumably because their gelatinous egg coverings are extremely transparent to UV. Likewise, the egg mass jelly of the dorid nudibranch *Archidoris montereyensis* provides embryos with little protection from mortality associated with exposure to direct solar radiation, although embryos embedded deeper within these egg masses do experience higher survival relative to peripheral embryos (Biermann et al., 1992). Clearly, the differences among these studies may be associated with variation in the material composition of these egg masses relative to *Nucella* capsules. Within one subclass of the Gastropoda alone, egg coverings can vary substantially in morphology (i.e., size, shape, surface texture), chemical composition, and structure (i.e., leathery vs. gelatinous), yet the relative merits of these structures as shields against UV are unknown. Likewise, the protective nature of capsule masses may also reflect the exposure of developing eggs to ultraviolet radiation within the habitats in which eggs are spawned. Increased exposure to solar radiation is correlated with the increased concentration of UV-absorbing compounds or DNA repair enzymes in many aquatic organisms (Dunlap et al., 1986; Karentz et al., 1992; Blaustein et al., 1994). Clearly, therefore, more research is necessary to understand how these factors may influence the spectral properties of egg masses of intertidal and shallow water aquatic organisms.

Although *Nucella* capsules can prevent >95% of incident UV-B radiation from crossing the capsule wall, the mechanism by which capsules shield embryos from UV remains unclear. Some radiation is undoubtedly scattered at the capsule surface. In terrestrial plants, for instance, leaf surface reflectance provides a first line of defense against UV, however, the surface scattering of light is generally < 10% of the incident radiation for most plants (Gausman et al., 1975; Robberecht et al., 1980). Although the attenuation of UV in the surface layers of *Nucella* capsule walls likely resulted from both absorption and surface reflectance (see Day et al., 1992), given the profiles of UV transmittance deeper in the capsule wall, surface scattering is unlikely to be extensive relative to the absorption of radiation by the capsule wall itself. In fact, the effectiveness of *Nucella* capsules as shields from UV appears to depend on the thickness of the outer capsule walls. *Nucella emarginata* capsules collected from Grappler Inlet and Kirby Point sites, where snails typically lay thicker-walled egg capsules (Rawlings, 1994), absorbed significantly more UV-A and UV-B radiation than thinner-walled capsules from Ross Islet. Interspecific differences in the spectral properties of *Nucella* capsules also reflected the same trend. Egg capsules of *N. lamellosa,* which are significantly thinner-walled than those of *N. canaliculata* and *N. emarginata* (Rawlings, unpub. data), were also significantly more transparent to UV. Interestingly, the relative transmittance of the epidermal tissue of terrestrial plants to UV is also inversely correlated with the thickness of the epidermis (Day, 1993). The absorption of UV by *Nucella* capsule walls was not associated with any particular region of the outer capsule wall, however, even though the microstructure of the outer capsule wall varies with depth (Rawlings, 1990). These results thus suggest that the UV-absorbing properties of *Nucella* capsules are associated with some general component of the capsule wall itself.

Despite the prevalence of mycosporine-like amino acid compounds as a biochemical defense against UV in many marine organisms (Karentz et al., 1991a), the absorption of UV in the egg capsules of *Nucella emarginata* was not associated with the presence of MAAs. These UV-absorbing compounds are easily extracted from minced tissues soaked in methanol (either freeze-dried tissue as in Karentz et al., 1991a, or wet tissues, as in Shick et al., 1992). In the present study, capsule pieces were left in methanol for over 10 times the length of time required for 99% extraction of MAAs from minced tissues (Karentz et al., 1991a), yet the transmittance of UV radiation across the capsule wall was unaffected by methanol treatment. Interestingly, although MAAs have been extracted from a variety of Antarctic marine gastropods, including the muricid snail, *Trophon* cf.

geversianus, attempts to extract MAAs from the egg capsules of *Trophon* have proved fruitless (Karentz et al., 1991a). Also, only trace amounts of one MAA were found in the benthic egg ribbons of an unidentified species of nudibranch examined (Karentz et al., 1991a). A variety of MAAs have been found in the eggs of limpets and fish species with planktonic development (Chioccara, et al., 1980; Karentz et al., 1992), however, perhaps reflecting differences in the exposure of larvae to UV during pelagic development versus benthic encapsulated development (see Karentz, 1994). Hence, benthic egg capsules and masses of marine gastropods do not appear well endowed with MAA defenses against UV exposure.

What then is responsible for absorbing UV radiation in the walls of *Nucella* egg capsules? Interestingly, the UV-absorbing properties of these capsules may be associated with compounds involved in the cross-linking of capsule proteins (see Price and Hunt 1973, 1974, 1976). Neogastropod egg capsules are composed mainly of protein and carbohydrate (Bayne, 1968; Flower et al., 1969; Hunt, 1966; Hunt, 1971; Flower, 1973; Price and Hunt, 1973; Sullivan and Bonar, 1984; Colman, and Tyler, 1988; Hawkins and Hutchinson, 1988), yet the structural components of their walls have been difficult to characterize because capsules are resistant to a wide range of degradative and disruptive agents. In an attempt to understand the properties of egg capsules from the marine subtidal gastropod *Buccinum undatum*, Price and Hunt (1973) first noted that these capsules fluoresced a blue-white light when exposed to UV radiation. Subsequent investigation indicated that this fluorescence resulted from a yellow fluorophore covalently bound to peptides within the capsule wall (Price and Hunt, 1974). Interestingly, although this fluorophore was not associated with material secreted by the capsule gland (Price and Hunt, 1976), capsules did fluoresce once they had been molded and hardened within the ventral pedal gland (Price and Hunt, 1976).

Although the mechanism by which the ventral pedal gland hardens capsules is not clear, this gland probably secretes a compound involved in the crosslinking of capsule proteins (see Price and Hunt, 1976). Examinations of both the fluorophore and secretions of the ventral pedal gland suggest that this substance contains aromatic aldehydes and proteins, but further characterization has been unsuccessful (Price and Hunt, 1974, 1976). Additional evidence that this fluorophore may be a property of the cross-linking moiety of these proteins has been provided by the emission of blue-white fluorescence from a number of structural proteins of molluscs, arthropods and mammals when exposed to UV radiation (Price and Hunt, 1976, and references therein). Thus, although UV-fluorescing compounds have not been described for *Nucella* egg capsules specifically, given the yellow color of these capsules, and their similar chemical properties to *Buccinum* capsules, the UV-absorption of *Nucella* capsules may also result from the presence of fluorophores within the capsule wall.

Significance of the capsule wall as a barrier to UV radiation

Even though capsules may filter out a large proportion of incident UV radiation, the biological significance of this absorption remains to be determined. Although 95 - 99% of UV-B radiation may be filtered out by the capsule walls, 1 - 4% of this potentially lethal radiation still enters the chamber of the thickest *N. emarginata* capsules. Likewise, UV-A radiation has some deleterious consequences for aquatic organisms (e.g., Smith et al., 1992), yet its passage across *Nucella* capsule walls is relatively unhindered compared to UV-B radiation. Given these facts, can the capsule wall really be considered a biologically-significant barrier to UV?

Although difficult to answer, several lines of evidence indicate that the capsule walls of *Nucella emarginata* are indeed effective UV-filters. Rarely do barriers of UV-absorbing pigments or compounds ever absorb 100% of the incident radiation. In the terrestrial cyanobacterium, *Chlorogloeopsis sp.*, for instance, the pigment scytonemin, produced in response to elevated levels of UV-A, only absorbs 90% of the incoming UV-A radiation (Garcia-Pichel and Castenholz, 1991). However, this pigment is still sufficient to increase the resistance of cultures to photoinhibition of photosynthesis (Garcia-Pichel, et al., 1992). Likewise, measurements of the transmittance of ultraviolet radiation across the foliage of evergreen and deciduous plants have shown that, on average, 4% and 28% of UV-B radiation can penetrate the mesophyll layer containing the photosynthetic machinery of evergreens and deciduous species, respectively (Day, 1993). These high transmittances occur despite the presence of UV-absorbing compounds within the epidermis. Hence, absorption levels of UV-B radiation by *Nucella* egg capsules are certainly consistent with the role of a UV-filter.

The results of both outdoor experiments using stripped and whole egg capsules of *Nucella emarginata* further corroborate this conclusion. Although these experiments probably do not reflect natural dosages and durations of exposure of capsule to UV, they do serve to illustrate an important point. The absorption of UV by *Nucella* capsule walls can clearly mean the difference between survival and death for encapsulated embryos. Embryos devoid of this barrier suffer substantially higher mortality when exposed to full spectrum solar radiation compared to embryos within intact capsules, or those in stripped capsules not exposed to UV. Although some embryos died in whole capsules exposed to UV in Experiment II, this difference among experiments could reflect variation in the source population of the egg capsules (i.e., thick-walled lab-laid capsules vs. thin-walled

Ross Islet capsules for Experiment I and II, respectively), differences in the susceptibility of early vs. late staged embryos to UV, or simply differences in the duration and intensity of exposure to UV between these experiments. Nevertheless, stripped capsules exposed to UV exhibited higher mortality in both experiments. Hence, under these conditions, capsule walls did serve as a biologically significant barrier to UV radiation.

Natural levels of UV-A radiation may not affect the survival of *Nucella* embryos. Although UV-A radiation has a negative effect on photosynthetic organisms by causing photoinhibition in phytoplankton (e.g., Helbling et al., 1992; Smith et al., 1992, and references therein), and can significantly inhibit settlement by planula larvae of corals (Baker, 1995), the great variability of responses among marine organisms to UV-A exposure has led some to believe that this form of radiation is not a unique or very important environmental factor (Damkaer et al., 1980). In fact, exposure to UV-A may even be beneficial to many organisms. Some species are able to detect UV-A and use it as a cue for avoiding exposure to the more harmful UV-B radiation (Bothwell et al., 1994, and references therein). Likewise, the presence of UV-A radiation is critical to the successful photoenzymatic repair of DNA damaged as the result of exposure to UV-B radiation (e.g., Karentz et al., 1991b; Smith et al., 1992). Hence, exposure to UV-A radiation may be far less detrimental to non-photosynthetic marine organisms than exposure to UV-B radiation. Thus, this might explain the differential transmittance of UV-A and UV-B radiation across *Nucella* capsule walls.

Although the biological significance of UV-absorbing compounds has been demonstrated in many other marine organisms by positive associations between the concentrations of MAAs and the potential for exposure to UV (e.g., Dunlap et al., 1986; Shick et al., 1992), the extent to which intra- and interspecific differences in the spectral properties of *Nucella* capsules reflect their potential for exposure to UV radiation remains to be examined. Clearly, variation in the UV-absorbing properties of *Nucella lamellosa*, *N. canaliculata* and *N. emarginata* capsules is somewhat consistent with the frequency of exposure of egg capsules to UV radiation. Typically, *Nucella lamellosa* has a lower intertidal distribution than either *N. canaliculata* and *N. emarginata* (Bertness and Schneider, 1976; Palmer, 1980), thus resulting in a shorter duration of exposure of capsules to direct sunlight. Interestingly, capsules of *N. lamellosa* are also significantly more transparent to UV than the other two *Nucella* species examined here. Other potential differences in the reproductive biology of these species must also be considered (e.g., spawning season, spawning sites, and reproductive patterns), however, before these interspecific differences in the spectral properties of *Nucella* egg capsules can be interpreted clearly.

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Table 5-1. Non-significant subsets resulting from three separate Contingency table analyses comparing the frequency of habitats used for capsule deposition versus the frequency of those available. These a posteriori comparisons were conducted using a simultaneous test procedure. Imposed on these results were the rankings based on the frequencies of habitats used versus those available.

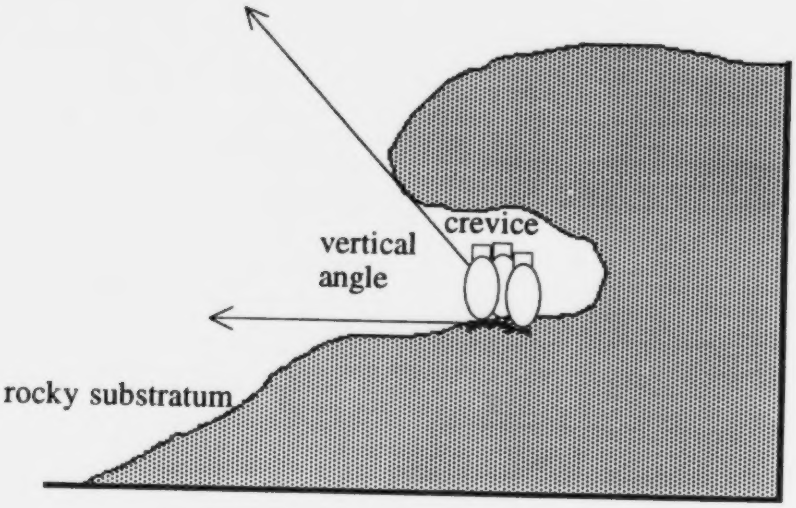
Site			**Habitat Preference**			
	Least Preferred				Most Preferred	
Ross Islet	Turf Algae	Balanus	*Bare Rock*	*Mytilus*	*Semibalanus*	
Wizard Rock	*Mytilus*	*Balanus*	*Pollicipes*	Turf Algae	Bare Rock	*Semibalanus*
Kirby Point	Bare Rock	*Balanus*	*Semibalanus*	*Mytilus*	Turf Algae	*Pollicipes*

Table 5-2. Percentage of embryos surviving within six whole and six stripped capsules when exposed to one of three different sunlight treatments in two separate outdoor experiments.

	Dark		**UV Filtered**		**UV Exposed**	
Experiment I	Whole (X ± S.E.)	Stripped (X ± S.E.)	Whole (X ± S.E.)	Stripped (X ± S.E.)	Whole (X ± S.E.)	Stripped (X ± S.E.)
Rep. 1	98.0±1.24	100.0±0.0	99.0±0.98	100.0±0.0	99.3±0.67	71.7±17.89
Rep. 2	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.9±1.12	64.4±20.48
Rep. 3	100.0±0.0	100.0±0.0	98.4±1.06	100.0±0.0	100.0±0.0	65.8±12.11
Rep. 4	100.0±0.0	100.0±0.0	98.1±1.34	100.0±0.0	99.3±0.72	31.0±9.08
Experiment II						
Rep. 1	97.0±1.96	83.3±16.70	93.8±6.25	84.9±8.75	46.4±20.98	32.5±20.56
Rep. 2	100.0±0.0	98.6±1.38	97.3±2.67	100.0±0.0	27.3±17.32	1.0±0.98
Rep. 3	92.9±7.15	80.0±20.00	100.0±0.0	100.0±0.0	78.9±15.90	33.3±21.08

Figure 5-1. Measurement of a) the vertical angle and b) the horizontal angle of exposure of two naturally-deposited groups of egg capsules of *Nucella emarginata* to direct solar radiation (see Fig. 5-7). Angles were measured using a modified protractor and map divider.

a) Side view:


crevice
vertical angle
rocky substratum

b)Top view:

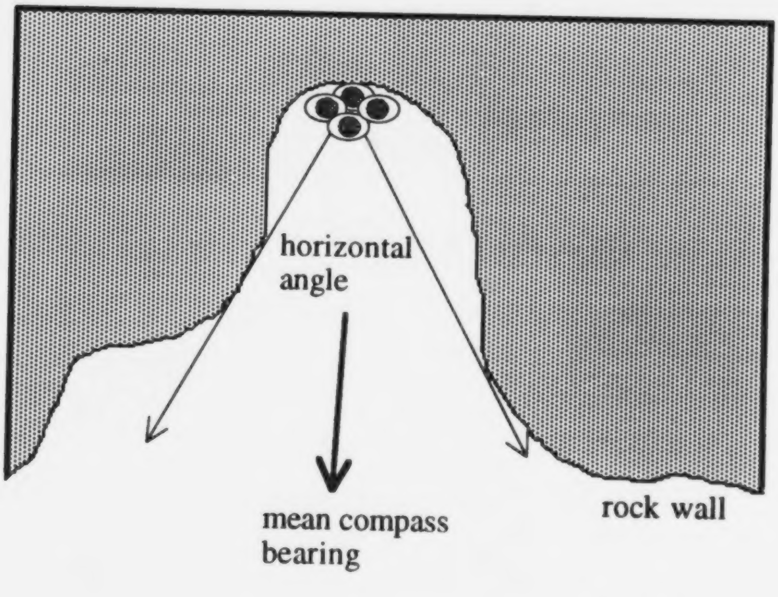

horizontal angle
mean compass bearing
rock wall

Figure 5-2. Spectral properties of UV-transmitting and UV-absorbing Acrylite plates used in Experiments I and II. Spectral properties were measured using a UV-visible Perkin-Elmer Spectrophotometer (Coleman 139).

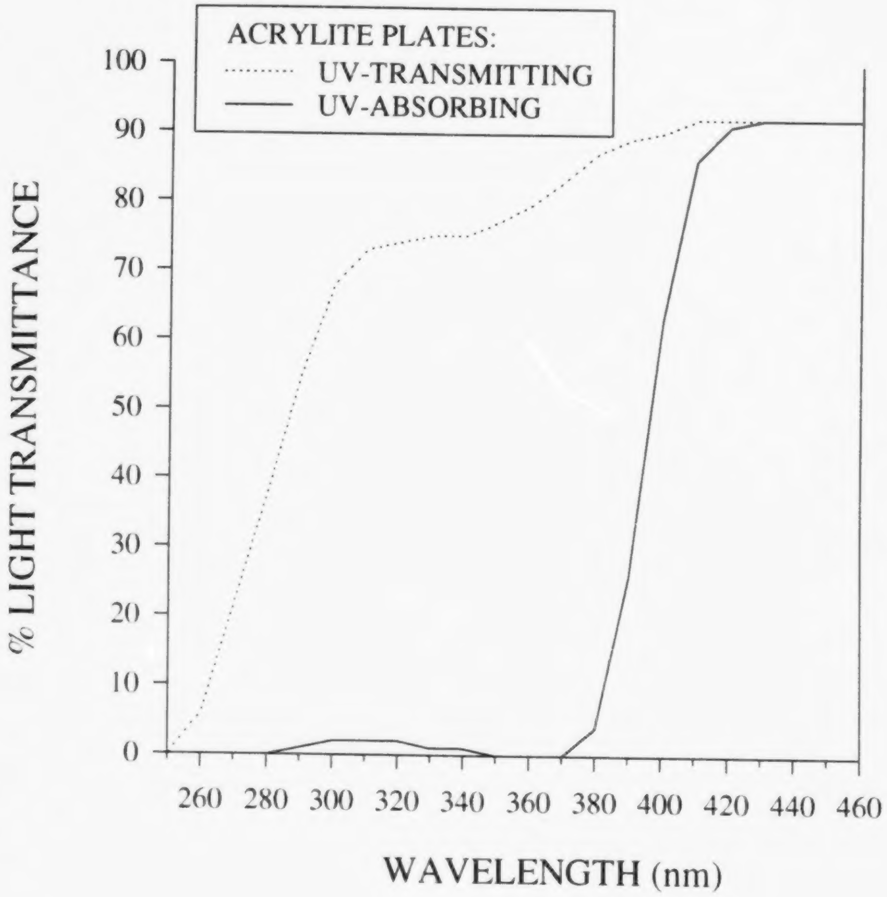


Figure 5-3. Diagram of the outdoor experimental set-up designed to examine the susceptibility of embryos within stripped and whole capsules to different sunlight treatments: a) Top view, illustrating the arrangement of treatment conditions and replicate groups, and b) Side view, showing the culture jars mounted within their opaque plastic trays and suspended in the seawater tank.

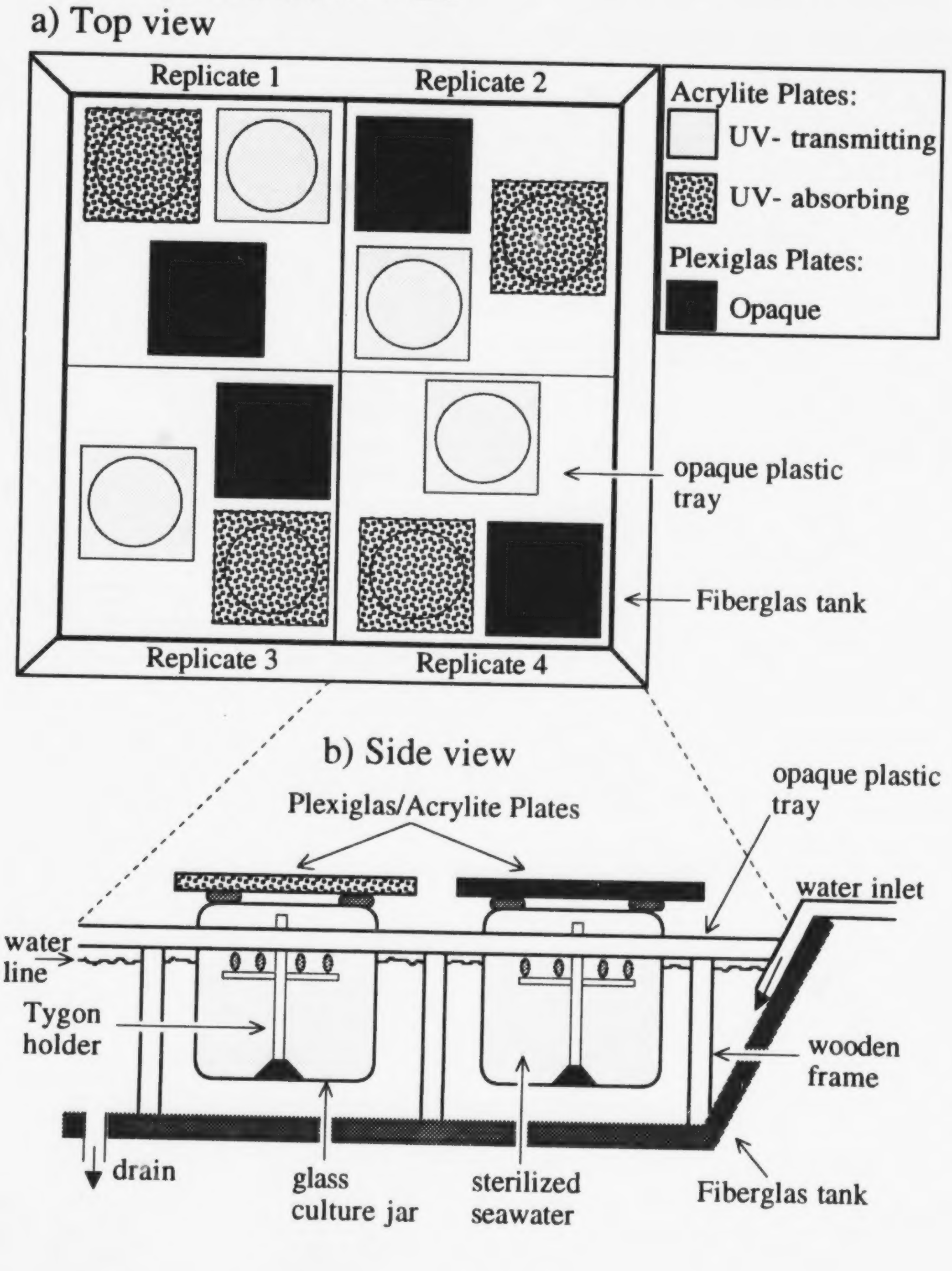

a) Top view
Replicate 1
Replicate 2
Replicate 3
Replicate 4
Acrylite Plates:
UV- transmitting
UV- absorbing
Plexiglas Plates:
Opaque
opaque plastic tray
Fiberglas tank
b) Side view
Plexiglas/Acrylite Plates
opaque plastic tray
water inlet
water line
Tygon holder
wooden frame
drain
glass culture jar
sterilized seawater
Fiberglas tank

Figure 5-4. Average percent cover (± SE) of the primary substratum by algal canopy and understorey microhabitats in 20 "available" quadrats and in 20 quadrats associated with *Nucella emarginata* egg capsules at Ross Islet, Wizard Rock, and Kirby Point. Understorey microhabitats were broadly categorized as: *Fucus* stipes (STIPES); turf algae (TURF ALG); bare rock (BARE RK); mussels, *Mytilus spp.*, (MYTILUS); barnacles, *Semibalanus cariosus* (SEMIBAL), *Balanus glandula* (BALANUS), and *Pollicipes polymerus* (POLLICIP); encrusting coralline algae (CORALL.); and anemones, *Anthopleura elegantissima* (ANTHOP). The dotted vertical line separates measures of percent cover of the algal canopy (left vertical axis) at each site from measures of percent cover of understorey habitats (right vertical axis). At all three sites, the frequency of microhabitats associated with *Nucella* egg capsules differed from the frequency of microhabitats that were present at each location (Contingency Table Analyses: **Algal canopy**: Ross: $G = 195.59$, $P < 0.001$; Wizard: $G = 199.52$, $P < 0.001$; **Primary Substratum**: Ross: $G = 242.09$, $P < 0.001$; Wizard: $G = 112.97$, $P < 0.001$; Kirby: $G = 143.84$, $P < 0.001$). Also included in this figure is the average percent cover (± S.E.) of algal canopy and understorey microhabitats in 20 quadrats associated with *Nucella emarginata* egg capsules at Execution Cave.

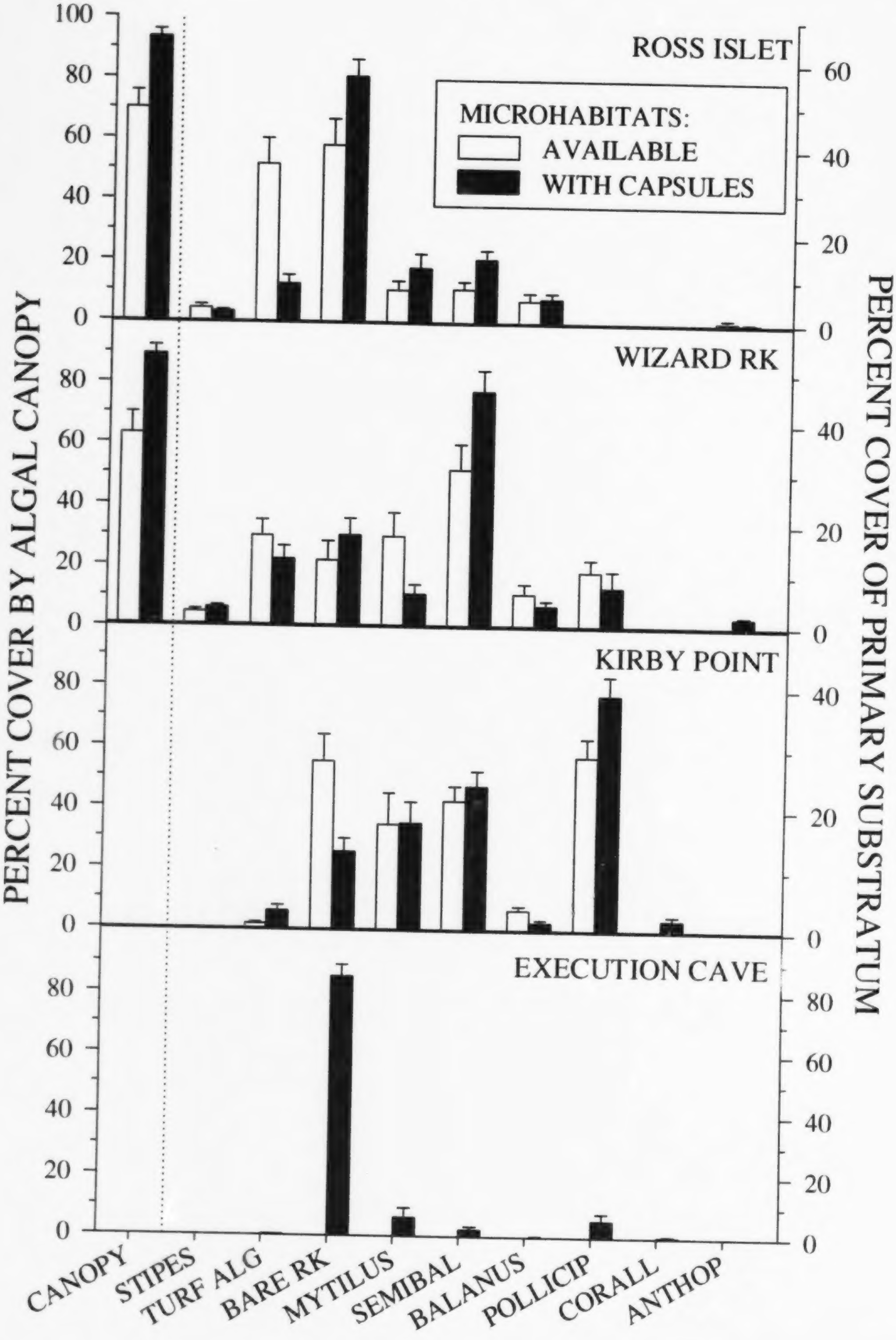

PERCENT COVER BY ALGAL CANOPY
PERCENT COVER OF PRIMARY SUBSTRATUM
ROSS ISLET
WIZARD RK
KIRBY POINT
EXECUTION CAVE
MICROHABITATS:
AVAILABLE
WITH CAPSULES
100
80
60
40
20
0
CANOPY
STIPES
TURF ALG
BARE RK
MYTILUS
SEMIBAL
BALANUS
POLLICIP
CORALL
ANTHOP

Figure 5-5. Percentage of capsules deposited in each of four different microhabitats by *Nucella emarginata* individuals at Ross Islet, Wizard Rock, and Kirby Point. The frequency with which these habitats were selected differed significantly among the three study sites (Contingency Table Analysis: G = 727.22, P < 0.001). Included for comparison are the habitats selected for capsule deposition at Execution Cave.

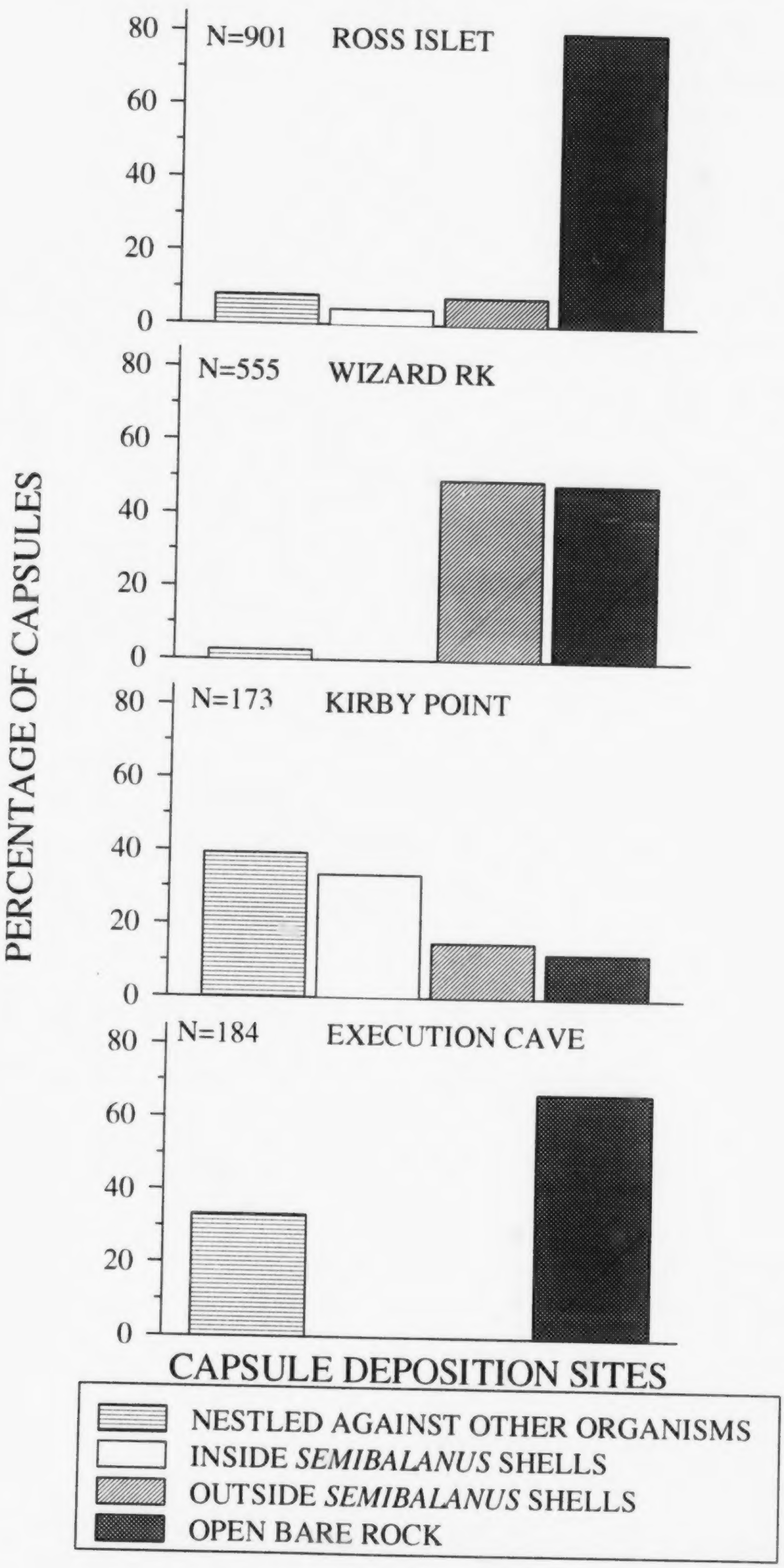

PERCENTAGE OF CAPSULES
N=901
ROSS ISLET
N=555
WIZARD RK
N=173
KIRBY POINT
N=184
EXECUTION CAVE
0
20
40
60
80
CAPSULE DEPOSITION SITES
NESTLED AGAINST OTHER ORGANISMS
INSIDE *SEMIBALANUS* SHELLS
OUTSIDE *SEMIBALANUS* SHELLS
OPEN BARE ROCK

Figure 5-6. A group of *Nucella emarginata* egg capsules deposited within an empty test of the acorn barnacle, *Semibalanus cariosus,* at Kirby Point in August 1994. Note also the dense covering of the substratum by the gooseneck barnacle, *Pollicipes polymerus,* and the lack of algal canopy.

Figure 5-7. Degree of vertical and horizontal exposure of naturally deposited groups of *Nucella emarginata* egg capsules to direct sunlight at Ross Islet (n = 20 groups), Wizard Rock (n = 20 groups), and Kirby Point (n = 17 groups). See Fig. 5-1 for an illustration of how these measurements were made. The results of a Contingency table analysis indicated that the extent to which capsules were exposed to direct solar radiation differed significantly among these sites: Vertical angle of exposure (frequency categories: 0 - 20°, 20 - 40°, 40 - 60°, 60 - 80°: $G = 41.47$, $P < 0.001$); Horizontal angle of exposure (frequency categories: 0 - 30°, 30 - 60°, 60 - 90°, 90 - 120°: $G = 45.11$, $P < 0.001$).

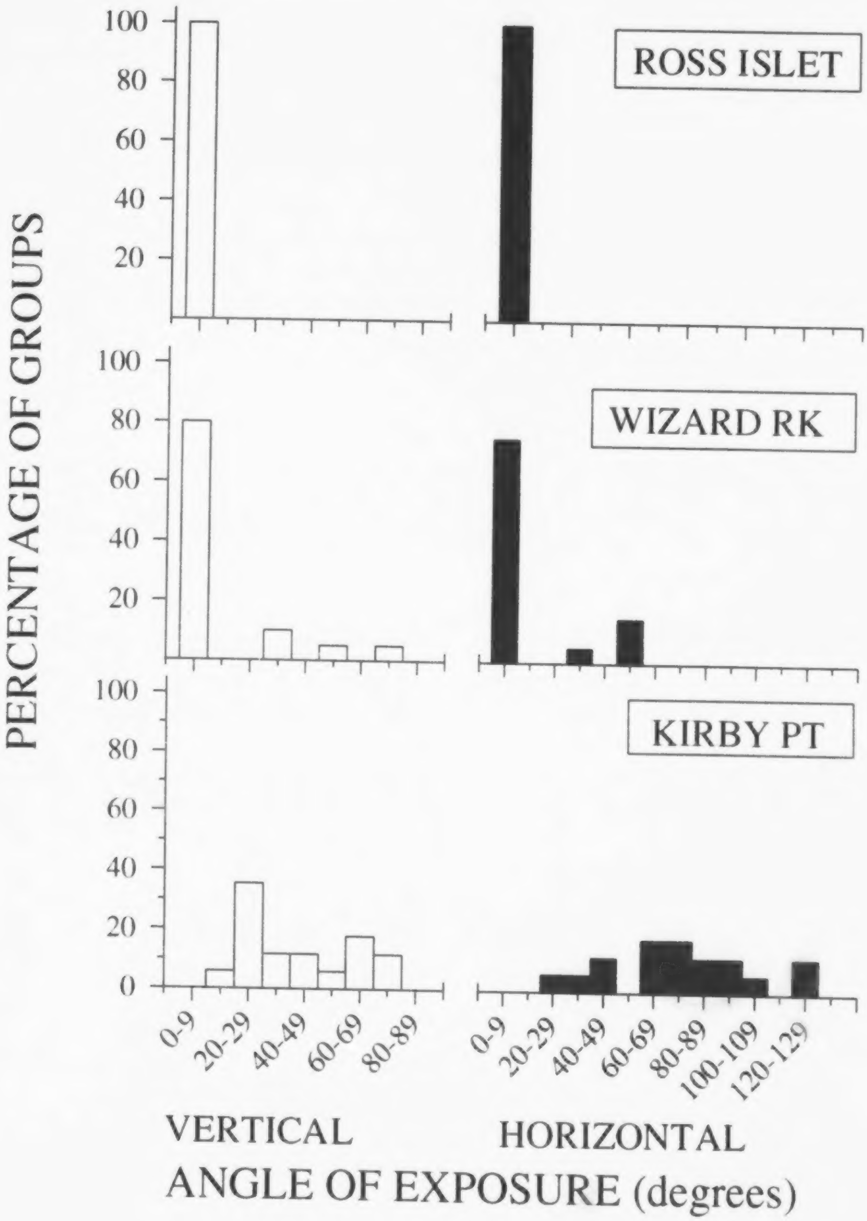


Figure 5-8. Variation in the spectral properties of the inner and outer wall of *Nucella emarginata* egg capsules (mean % transmittance ± SE; n = 3 capsules). Two separate t-tests (adjusted for heterogeneous variances) were used to compare the mean % transmittance of light across the inner and outer capsule wall laminae for representative wavelengths of UV-A (@ 360 nm) and UV-B radiation (@ 300 nm). Results of both analyses indicated that the inner capsule wall was more transparent to UV-A (360 nm: t-test, $t' = 9.114$, df = 2, $P < 0.02$) and UV-B (300 nm: t-test: $t = 12.580$, df = 2, $P < 0.01$).

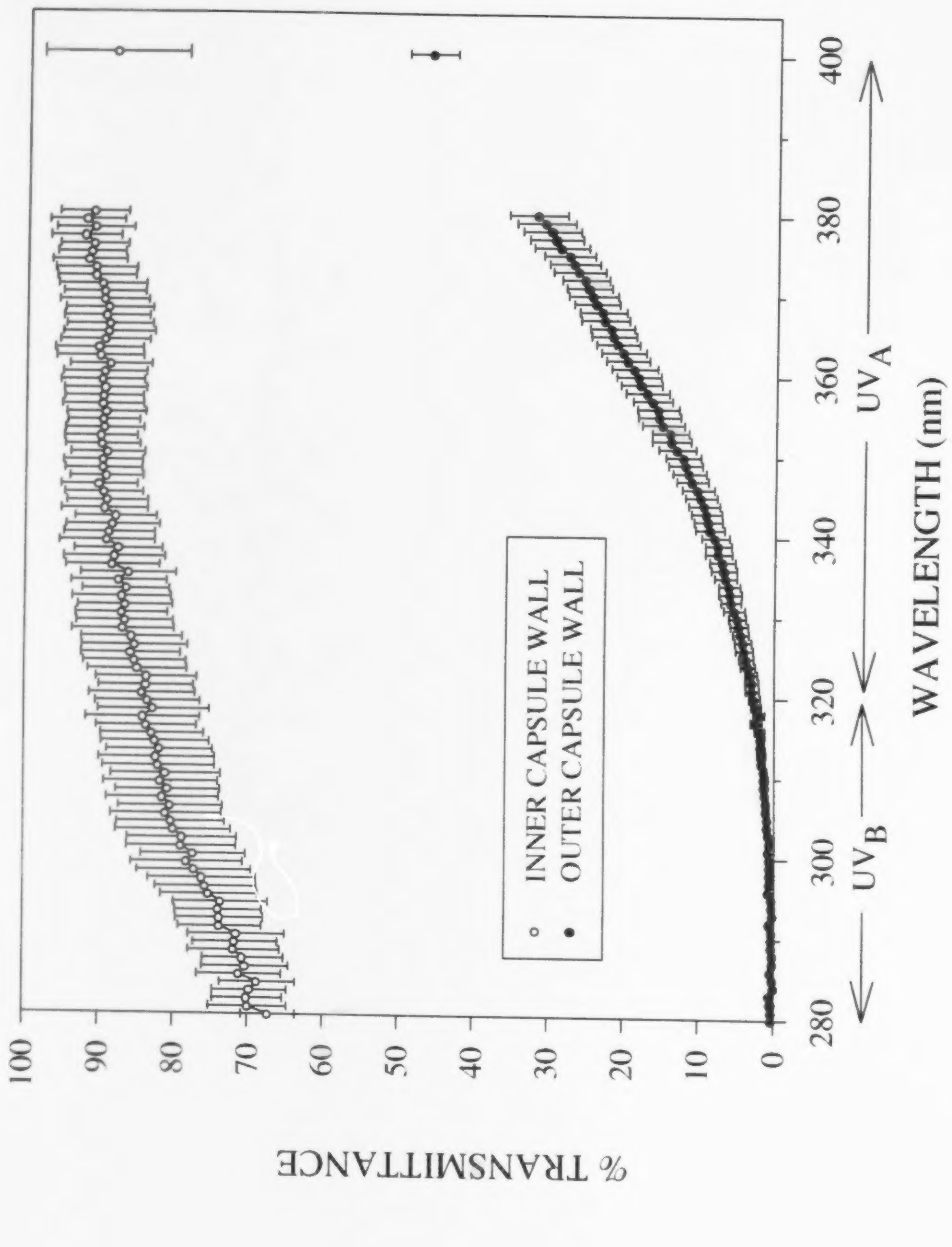

% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
280
300
320
340
360
380
400
WAVELENGTH (nm)
UV_B
UV_A
○ INNER CAPSULE WALL
● OUTER CAPSULE WALL

Figure 5-9. Variation in the spectral properties of egg capsules (mean % transmittance ± SE) among three populations of *Nucella emarginata*, based on a sample of eight capsules from each population. Percent transmittance of UV-A (representative wavelength @ 360 nm) and UV-B (representative wavelength @ 300 nm) radiation was compared among populations using a 2-level Nested ANOVA. These results indicated a significant effect of both source population and capsule for both wavelengths: UV-A : 2-level Nested ANOVA: F (population) = 3.234, df = 2, 21, $P \approx 0.07$; F(capsule) = 8.780, df = 21, 48, $P < 0.001$; UV-B: 2-level Nested ANOVA: F (population) = 7.357, df = 2, 21, $P < 0.01$; F(capsule) = 13.290, df = 21, 48, $P < 0.001$. *A posteriori* comparisons indicated that Ross Islet capsules were significantly more transparent to UV-A and UV-B radiation than either Grappler Inlet or Kirby Point capsules.

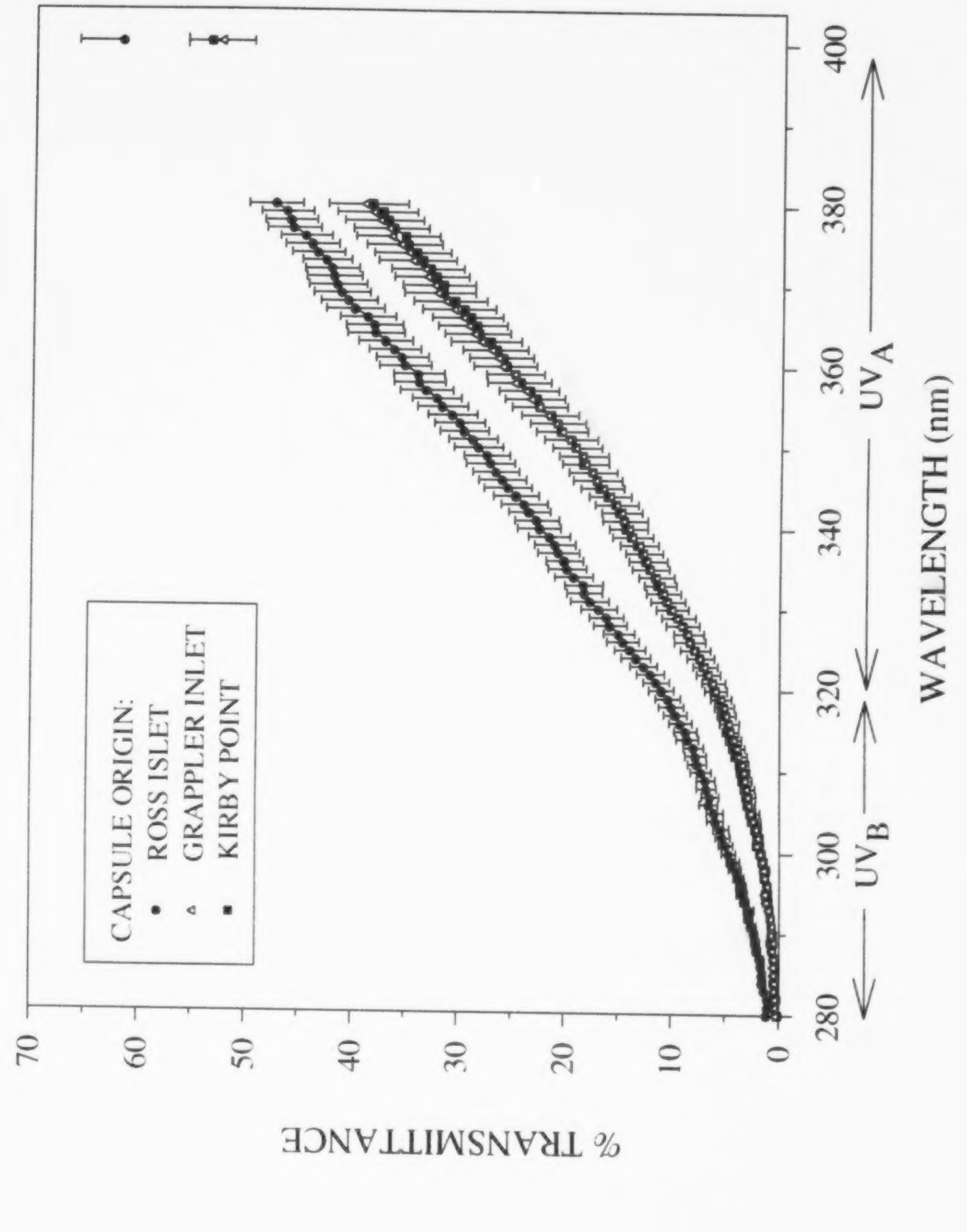

CAPSULE ORIGIN:
● ROSS ISLET
▵ GRAPPLER INLET
■ KIRBY POINT
% TRANSMITTANCE
0
10
20
30
40
50
60
70
280
300
320
340
360
380
400
WAVELENGTH (nm)
UV_B
UV_A

Figure 5-10. Comparison of the % transmittance of UV radiation across the capsule walls of *Nucella emarginata*, *N. lamellosa*, and *N. canaliculata* egg capsules (mean ± SE). Percent transmittance for UV-A (@ representative wavelength of 360 nm) and UV-B wavelengths (@ representative wavelength of 300 nm) were compared using a 2-level Nested ANOVA. Both source species and capsule origin had a significant effect for both wavelengths: UV-A: 2-level Nested ANOVA: F (species) = 8.75, df = 2, 21, $P < 0.01$; F(capsule) = 6.34, df = 21, 47, $P < 0.001$. UV-B: 2-level Nested ANOVA: F (species) = 44.62, df = 2, 21, $P < 0.001$; F(capsule) = 8.48, df = 21, 47, $P < 0.001$. *A posteriori* comparisons for both species indicated that *Nucella lamellosa* capsules were significantly more transparent to UV-B and UV-A radiation than either *N. canaliculata* and *N. emarginata* capsules; no significant difference was found between *N. canaliculata* and *N. emarginata* capsules.

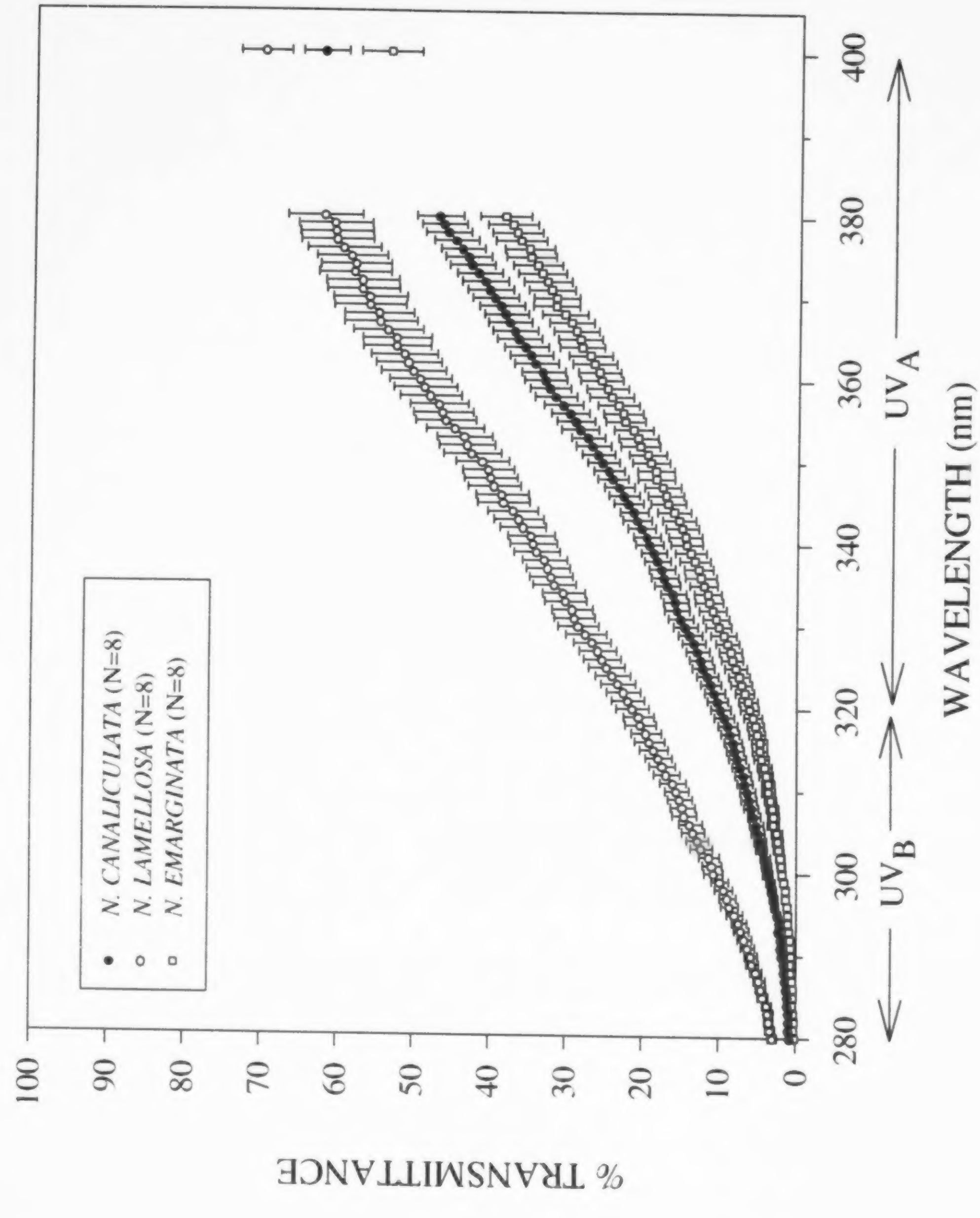

% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
280
300
320
340
360
380
400
UV$_B$
UV$_A$
WAVELENGTH (nm)
● N. CANALICULATA (N=8)
○ N. LAMELLOSA (N=8)
□ N. EMARGINATA (N=8)

Figure 5-11. a) Percent transmittance of UV (mean ± SE; @ 300 nm) at various depths through the outer wall of *Nucella emarginata* capsules (n = 3). Capsules were either mounted forwards (hollow symbols) or backwards (filled symbols) with respect to the light source to determine if there was any polarity to the UV-absorbing properties of the outer wall (see inset diagrams: "O" refers to the outer surface of the wall; "I" refers to the inner surface of the wall). b) Average percent transmittance of UV at various distrances (in μm) from the outer surface of the walls of *N. lamellosa* (n = 1), *N. canaliculata,* and *N. emarginata* capsules (n = 3).

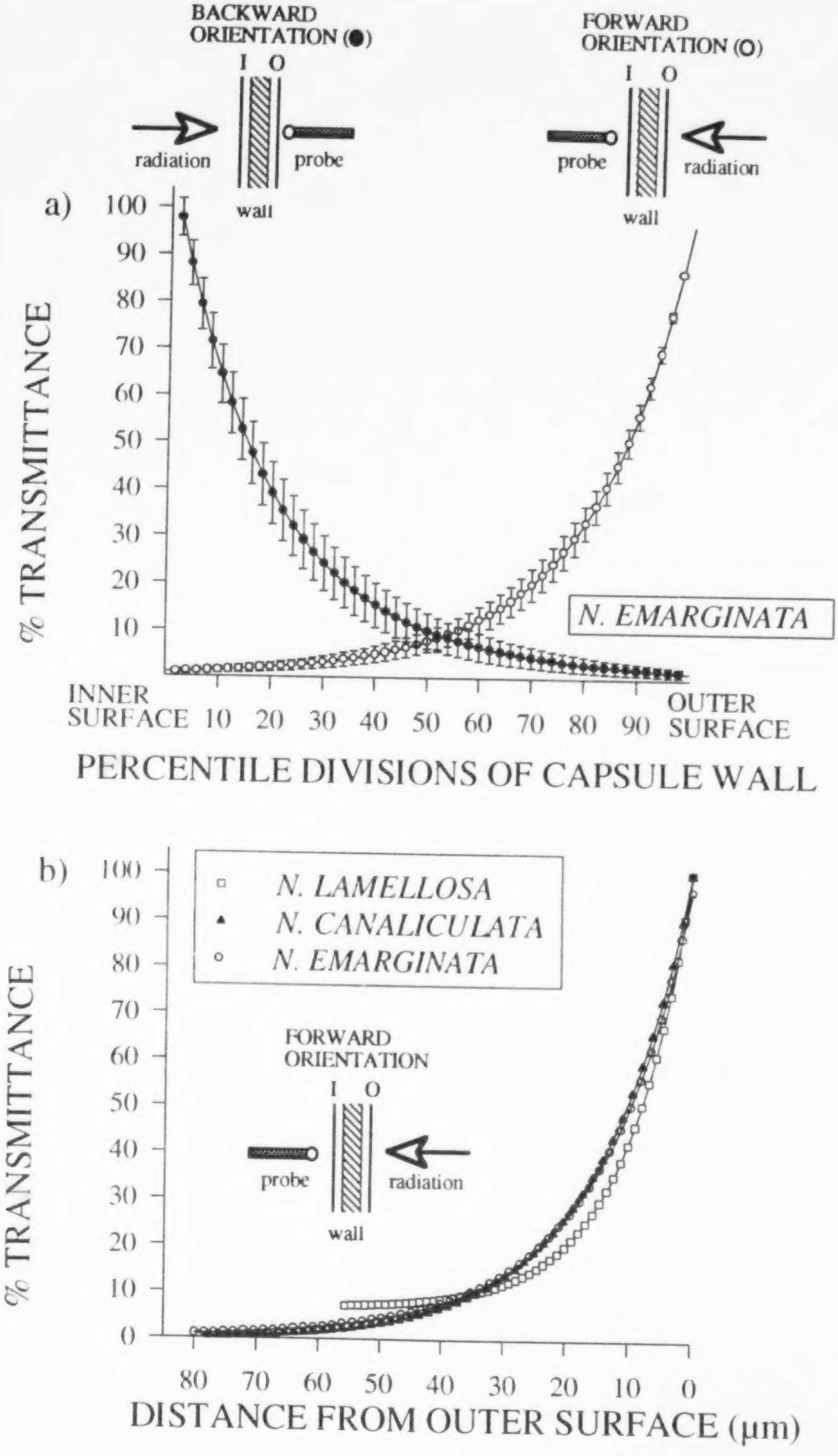

a)
BACKWARD ORIENTATION (●)
I O
radiation
probe
wall
FORWARD ORIENTATION (○)
I O
probe
radiation
wall
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
N. EMARGINATA
INNER SURFACE
10
20
30
40
50
60
70
80
90
OUTER SURFACE
PERCENTILE DIVISIONS OF CAPSULE WALL
b)
□ N. LAMELLOSA
▲ N. CANALICULATA
○ N. EMARGINATA
FORWARD ORIENTATION
I O
probe
radiation
wall
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
80
70
60
50
40
30
20
10
0
DISTANCE FROM OUTER SURFACE (μm)

Figure 5-12. Percent transmittance of UV radiation (mean ± SE) across the walls of *Nucella emarginata* capsule pieces ($n = 5$ paired pieces) soaked in seawater for 24 h versus those soaked in 80% methanol for the same period of time. A 2-factor ANOVA was used to compare the effect of extraction solvent (i.e., methanol vs. water) and capsule source on the transmittance of UV-A (@ 360 nm) and UV-B radiation (@ 300 nm) across the capsule wall. Solvent treatment had no significant effect on the transmittance of either UV-A or UV-B radiation, although was a significant difference in transmittance among capsules (UV- A, ANOVA: F (solvent) = 2.502, df = 1,4; $P > 0.10$; F(capsule) = 3.115, df = 4,20; $P = 0.04$; UV-B radiation, ANOVA: F (solvent) = 0.357, df = 1,4; $P > 0.50$; F(capsule) = 4.264, df = 4,20; $P = 0.01$).

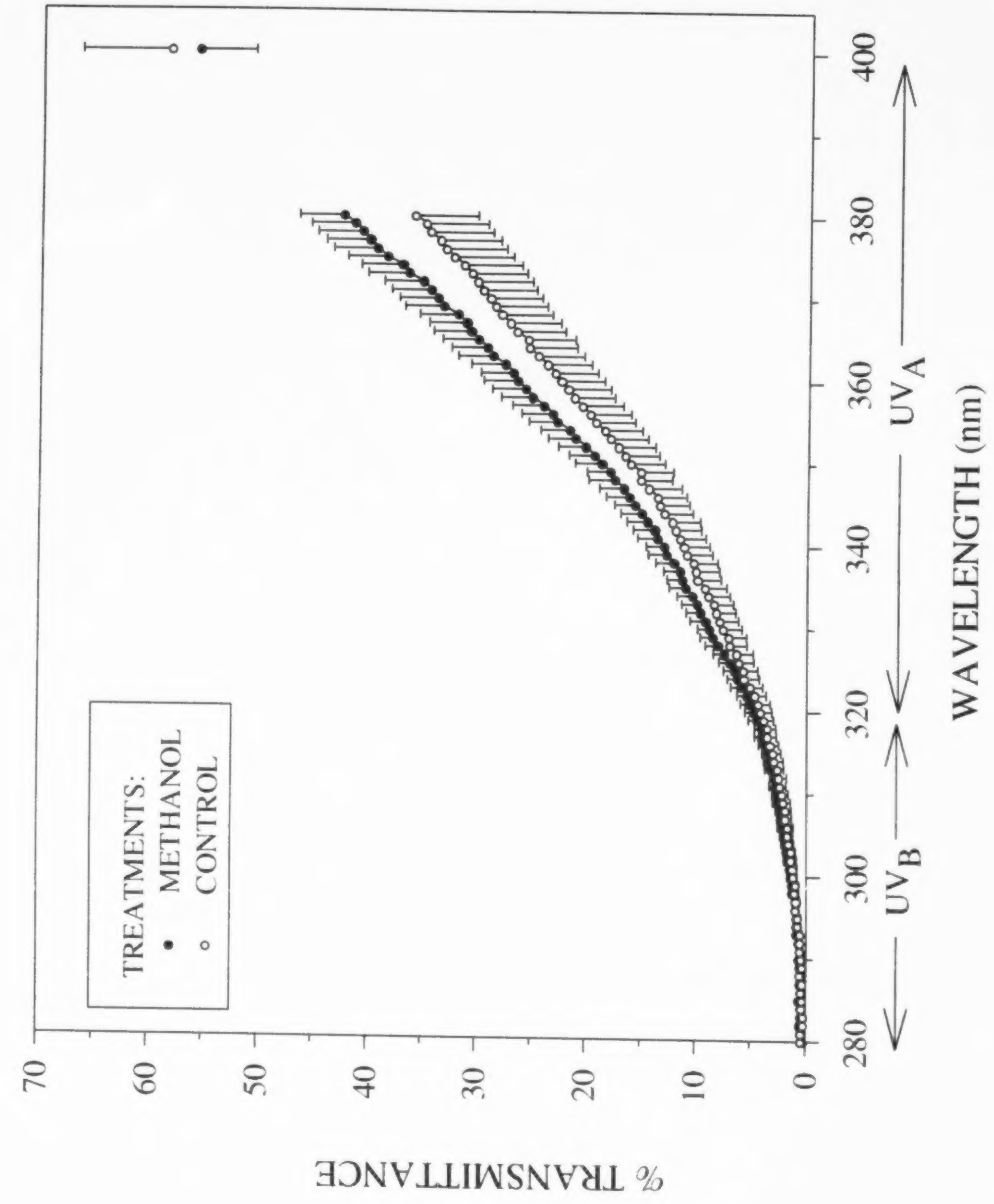

TREATMENTS:
● METHANOL
○ CONTROL
% TRANSMITTANCE
0
10
20
30
40
50
60
70
280
300
320
340
360
380
400
UV_B
UV_A
WAVELENGTH (nm)

Figure 5-13. Maximum midday UV-B intensity and the number of hours of sunshine per day during outdoor Experiment I (July 27 - Aug 10, 1993) and Experiment II (Aug 15 - Sept 6, 1993). Midday UV-B measurements were determined by taking the maximum UV-B intensity recorded at the Saturna UV Monitoring Station over the period from 11am - 1.30 pm for each day of these experiments. Daily hours of sunlight were recorded at the Tofino Weather Station. * refer to days where no UV-B information was available.

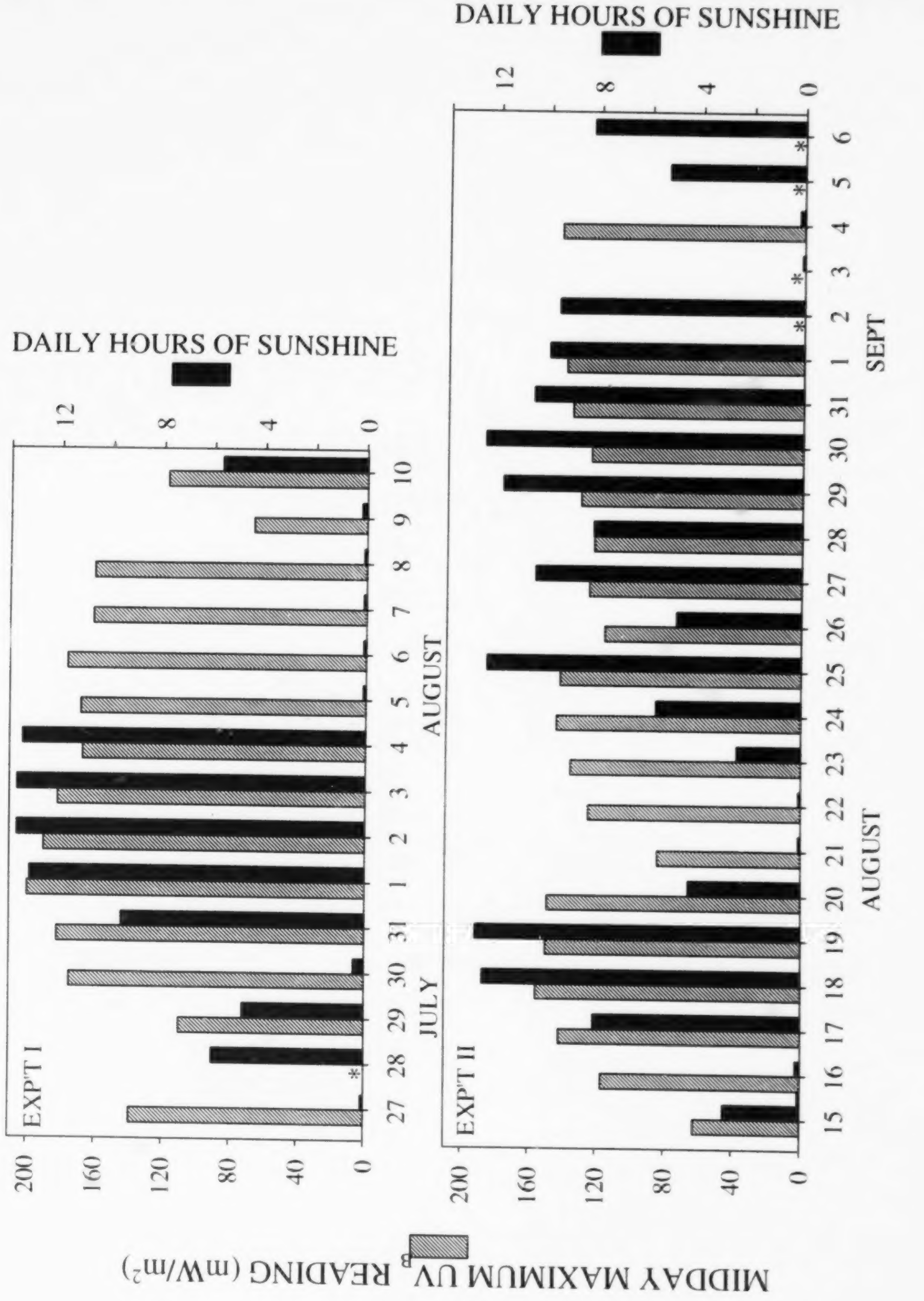
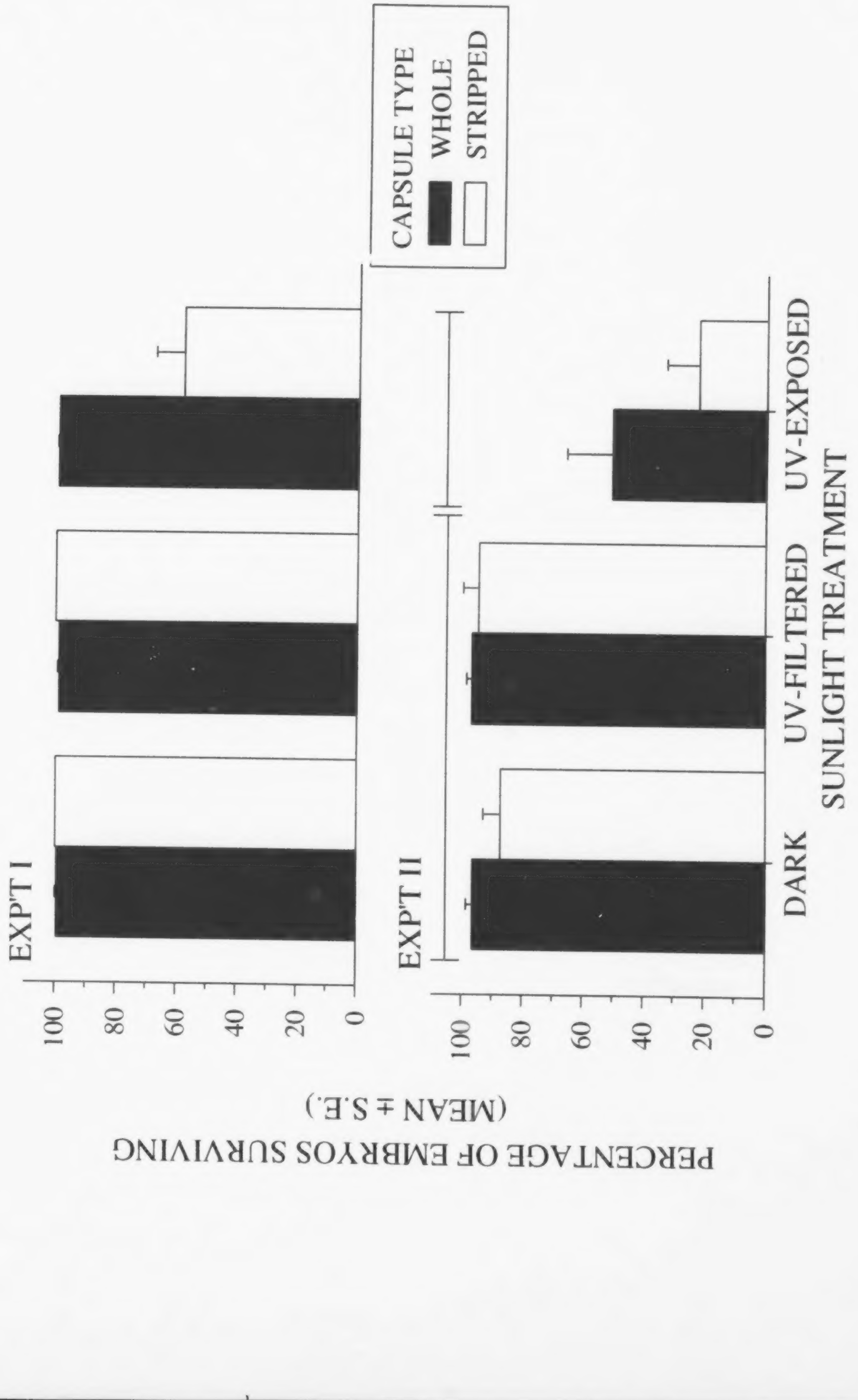

DAILY HOURS OF SUNSHINE
MIDDAY MAXIMUM UV$_B$ READING (mW/m^2)
EXP'T I
JULY
27 28 29 30 31
AUGUST
1 2 3 4 5 6 7 8 9 10
0 40 80 120 160 200
0 4 8 12
EXP'T II
AUGUST
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
SEPT
1 2 3 4 5 6
0 40 80 120 160 200
0 4 8 12
*

Figure 5-14. Percentage of embryos within stripped and whole egg capsules of *Nucella emarginata* that survived exposure to three different sunlight treatments in Experiement I and II. Capsules were exposed to treatment conditions of 15 and 23 days in Experiment I and II, respectively. Results of a two-way nonparametric analysis comparing the % of embryos surviving each treatment condition are as follows: Experiment I: χ^2 (cap wall) = 0.24, $P > 0.50$; χ^2 (sunlight) = 6.971, $P < 0.05$; χ^2 (interaction) = 8.091, $P < 0.025$. Experiment II: χ^2 (cap wall) = 0.333, $P > 0.50$; χ^2 (sunlight) = 12.150, $P < 0.01$; χ^2 (interaction) = 0.361, $P > 0.50$. Connecting horizontal lines above the results of Experiment II indicate non-significant differences among treatment conditions.


CAPSULE TYPE
WHOLE
STRIPPED
EXP'T I
EXP'T II
PERCENTAGE OF EMBRYOS SURVIVING
(MEAN ± S.E.)
100
80
60
40
20
0
DARK
UV-FILTERED
UV-EXPOSED
SUNLIGHT TREATMENT

CHAPTER 6

Direct observation of encapsulated development in muricid gastropods[1]

Abstract

Although muricid gastropods produce a spectacular array of benthic egg capsules, many aspects of their encapsulated development still remain poorly studied. This may be due in part to the fact that the capsule walls of many species are opaque, and attempts to rear early-stage embryos outside these structures have proven to be difficult. I describe a technique that allows developmental changes in the behavior and morphology of muricid embryos to be observed through the inner capsule wall. By selectively removing the thick, and often opaque, outer wall laminae of muricid egg capsules, embryos may be observed through the remaining transparent inner wall. Although embryos within stripped capsules are still vulnerable to contamination by protists and bacteria, embryos of the marine gastropod *Nucella emarginata* ("northern species") have been successfully reared in stripped capsules when cultured under sterile conditions. Hence, this technique provides a window through which to view the entire intracapsular development of muricid gastropods.

Stripped capsules may also be used to examine specific benefits and costs associated with the enclosure of embryos within benthic capsules. Because stripped capsules lack the thick, protective outer wall laminae, embryonic development within stripped and whole capsules can be compared to assess (1) the benefits of these laminae in protecting embryos from specific sources of mortality, and (2) their costs in terms of limiting the diffusion rate of oxygen and metabolic wastes into and out of the capsule chamber. Thus, this technique also has the potential to be an invaluable tool in helping to understand the role of encapsulating structures in gastropod life-histories.

Introduction

A wide variety of organisms enclose their eggs within some form of protective covering. These structures vary tremendously in form, ranging from the calcareous egg shells of many terrestrial vertebrates to the flexible, proteinaceous capsules of higher prosobranch gastropods. Because egg coverings are often thick-walled, composed of multiple laminae, and partially opaque, numerous techniques have been employed to investigate the development of embryos within these structures. Such techniques have often involved the chemical or mechanical removal of portions of egg coverings in order to observe embryonic development more clearly (e.g., avian egg shells: Tolhurst, 1974) or to determine the resistance of specific laminae to solute transport, thermal stress, and desiccation (insect egg cases: McFarlane, 1978; spider cocoons: Hieber, 1985; 1992). Other methods have involved the physical removal of embryos from their encapsulating structures in order to manipulate embryonic development experimentally or to determine the ability of embryos to develop outside these protective confines (e.g., gastropod capsules: Clement, 1952; Perron, 1981; Pechenik et al., 1984; Lord, 1986). These techniques have not only increased our understanding of embryonic development within opaque egg coverings, but have also helped to identify the role of encapsulating structures in protecting embryos from specific sources of mortality.

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[1] A version of this chapter has been published. Rawlings, T. A. 1995 Direct observation of encapsulated development in muricid gastropods. Veliger 38: 54 - 60.

Marine gastropods within the family Muricidae are typical of most higher prosobranch snails in that they enclose developing embryos within elaborate, structurally-complex, benthic egg capsules. Embryos remain within these capsules for varying portions of their development before emerging as either planktonic larvae or juvenile snails (D'Asaro, 1991). Although numerous studies have described sequential stages in the morphogenesis of muricid embryos (e.g. D'Asaro, 1966; Gallardo, 1979; Roller & Stickle, 1988), few have documented changes in the growth and behavior of embryos during their encapsulated development. This may be because capsule walls of many species are opaque, and the few attempts to rear early-stage embryos outside these structures have proven to be difficult (Pechenik et al., 1984; Stöckmann-Bosbach, 1988; but see Lord, 1986). Also, since many encapsulated embryos are enclosed with nutritive reserves, such as nurse eggs (Spight, 1976; Gallardo, 1979) and albumen (D'Asaro, 1966; Rivest, 1986; Stöckmann-Bosbach & Althoff, 1989), observations made on embryos cultured in an artificial fluid environment may not accurately reflect natural development within the egg capsule.

Here I describe a simple technique for removing the opaque outer capsule wall that not only enables the entire embryonic development of muricid snails to be followed within the egg capsule, but also allows embryos to remain within their natural medium. Hancock (1956) and Ganaros (1958) first indicated that the outer capsule wall of the muricid snail *Urosalpinx cinerea* (Say, 1822) could be peeled to allow direct observation of the embryos within. To my knowledge, however, this technique has never been described, nor has it been used to follow the developmental sequence of embryos over time. The laminated nature of muricid egg capsules makes itself amenable to this type of manipulation. Egg capsules within the family Muricidae are typically composed of three to four discrete laminae (Fig. 6-1; see also D'Asaro, 1988; Roller & Stickle, 1988): an outer protective lamina , L_1 ($\leq 5\mu m$), which seals the whole capsule including the escape aperture; a thick middle lamina, L_2 (usually $<100\mu m$), which comprises the internal skeleton of the capsule wall and is often composed of multiple fibrous layers; and one or two inner laminae, L_3, and L_4 (collectively $< 6\mu m$; D'Asaro, 1988), often not distinct from one another at the level of light microscopy, but which act to enclose the developing embryos and intracapsular fluid within a transparent chamber. The technique described herein relies on the separation of the outer capsule wall laminae L_1 and L_2 (hereafter collectively termed the "outer capsule wall"; Fig. 6-1) from the thinner innermost laminae L_3 and L_4 (hereafter collectively termed the "inner capsule wall"; Fig. 6-1). Although the following tests of this method were conducted on egg capsules of the muricid snail, *Nucella emarginata* (Deshayes, 1839), this method has also been used on capsules of *N. lamellosa* (Gmelin, 1791; Fig. 6-1) and *N. canaliculata* (Duclos, 1832), and should be applicable to other muricid capsules with a similar type of capsule wall microstructure.

Materials and Methods

Stripping egg capsules of *Nucella emarginata*

Freshly-laid egg capsules were collected from laboratory-raised females of the "northern species" of *Nucella emarginata* (see Palmer et al., 1990). The outside surface of each capsule was sterilized prior to removing portions of the outer capsule wall by briefly swabbing the capsule exterior with absorbent cotton soaked in 70% ethanol. Exposure to alcohol also caused capsules to dehydrate rapidly. This process aided in the separation of the outer capsule wall from the capsule chamber (see below), and did not appear to have any adverse effects on embryonic development. It should be noted, however, that the use of 70% ethanol was not essential for the successful stripping of capsules and subsequent culture of encapsulated embryos.

The outer capsule wall was stripped away from the underlying inner capsule wall using a disposable Reichert Histostat microtome blade. The initial cut into the capsule wall was made at an angle approximately 15° to the capsule surface, near the junction of the capsule chamber and plug, and at the region of the capsule seam; the capsule wall is usually slightly thickened along the length of the capsule chamber in the region of both seams.

Once the initial incision was made, the buckling outer capsule wall tended to return to its normal shape, leaving the inner wall deflated due to the evaporation of water from the chamber. As the capsule continued to lose water, the inner wall gradually peeled further away from the surface of the outer wall, thus allowing the outer wall to be removed piecemeal with little chance of rupturing the underlying chamber. Once approximately two-thirds of the outer wall had been removed on one side of the capsule, the capsule was repositioned and the process repeated on the opposite side. At the end of this, two panels of approximately equal size had been removed from the outer capsule wall. Although the entire outer wall could be removed following this technique, I elected not to do this since capsules are more easily damaged during handling without a portion of the outer wall or stalk for structural support. Once capsules were stripped, they were then placed in petri dishes of 1 μm filtered, autoclaved, antibiotic-treated seawater (0.050 g/l streptomycin; 0.030 g/l penicillin) for 1 d to determine if there were any leaks in the capsule chamber. Because the intracapsular fluid of freshly-laid *Nucella* capsules is extremely viscous (Pechenik et al., 1984; Stöckmann-Bosbach & Althoff, 1989; Rawlings, personal observation), any tears in the inner capsule wall were evident from the discharge of this fluid from the capsule chamber into the surrounding seawater.

Culture conditions

Since embryos within all stripped capsules failed to survive when cultured in 1μm filtered flowing seawater, I attempted to minimize the exposure of capsules to bacteria and protists by raising capsules in (a) sterile glass petri dishes containing 1μm filtered autoclaved, antibiotic-treated seawater, and (b) flowing, 1μm filtered, ultraviolet (UV) sterilized seawater. For each treatment, pairs of capsules were collected from laboratory-laid clutches of egg capsules. One capsule from each pair was then "stripped", as described above, and the other capsule was left intact ("whole" capsules).

In the treatment using autoclaved, antibiotic-treated seawater, each pair of capsules was assigned to one glass petri dish, and capsules were cultured in an incubator at 12°C. Glass dishes and seawater were changed at approximately 5 d intervals, at which time embryos were examined for development. Embryos were considered to have died when they turned pink, ceased to move their velar cilia, or began to disintegrate within the capsule.

In the UV sterilized seawater treatment, each pair of capsules was placed in holders made from Tygon™ tubing (Fig. 6-2). Stripped and whole capsules from the same clutch were placed side by side in Tygon™ holders by inserting their stalks into small slits perpendicular to the length of the tubing. Each Tygon holder was attached to the base of a 10 L culture chamber using a glass rod and suction cup, thus keeping capsules suspended at a height of approximately 0.1 m from the bottom of the chamber. In this manner, capsules were exposed to flow rates of 2 L/min of UV sterilized water, and seawater temperatures ranging from 10 - 12°C. Survival of encapsulated embryos was monitored at 4-8 d intervals, at which time capsules were also gently swabbed to remove any surface debris. Capsule holders were changed at two-week intervals.

Results

Embryos completed development successfully within 53% of 32 stripped capsules exposed to autoclaved, antibiotic-treated seawater, and 40% of 58 stripped capsules exposed to UV sterilized seawater (Table 6-1) over development times ranging from 52 to 77 days at 10 - 12°C. In those capsules in which embryos successfully hatched, no significant differences were evident in the number of hatchlings emerging from paired stripped and whole capsules (Paired t-test: $t = 1.52$, $P = 0.15$; $n = 15$ pairs [UV treatment, September, 1993]), with means (±S.E.) of 12.3 ±.1.80 and 10.4 ± 1.66 embryos hatching from stripped and whole capsules, respectively. The whole course of embryonic development could be observed through the transparent inner wall of stripped egg capsules (Fig. 6-3), including such events as: the initial reduction in viscosity of the capsular fluid; the ciliation and movement of the early-stage veligers; the onset and termination of feeding on nurse eggs; the development of such structures as the larval kidneys, adult kidney, foot, operculum, tentacles, and eyes; the resorption of the velar lobes; and the dissolution of the capsular plug. These observations paralleled morphological descriptions of encapsulated development in *Nucella emarginata* by LeBoeuf (1971) and Lyons & Spight (1973), based on the removal of embryos from their capsules. The photographs in Figure 6-3 are thus the first to document the natural *intracapsular* development of *Nucella emarginata* embryos.

Embryos developing within stripped capsules suffered considerably higher mortality than embryos in whole capsules under both culture conditions (Table 6-1; Fig. 6-4). Although embryos within all capsules reared in autoclaved, antibiotic-treated seawater survived in September 1992 (Table 6-1), under the same conditions in July 1993 there was a substantial increase in the mortality of embryos within stripped capsules. This appeared to be due to a flagellated protist that infected many cultures a few weeks after capsules had been stripped; these protists were never seen in my earlier 1992 cultures. Protists produced a mucous covering around the capsule and eventually managed to penetrate the inner capsule wall and feed on the developing embryos. Attempts to kill these organisms by periodically swabbing the exterior of infected capsules with 70% alcohol proved unsuccessful. The source of these protists was unclear, but it appears possible that protists were present on the capsule wall prior to stripping, and survived the initial swabbing with alcohol.

Embryos within stripped capsules also suffered higher mortality compared to those in whole capsules when cultured in UV sterilized seawater (Table 6-1, Fig. 6-4). Again, mortality of these embryos appeared to result from the penetration of the capsule chamber by protists.

Discussion

Attempts to rear neogastropod embryos outside their egg capsules have met with mixed success (Pechenik et al., 1984; Rivest, 1986). Embryos with relatively short encapsulated development times and a pelagic larval stage (e.g. *Ilyanassa obsoleta*; Costello & Henley, 1971; *Conus spp.*, Perron, 1981) appear to survive well when cultured outside the confines of their capsules walls. However, those with longer term development, and enclosed with nurse eggs for nutrition, have typically suffered much higher mortality when removed from the capsule chamber. Pechenik et al. (1984) reported 95% mortality of early stage "excapsulated" embryos of muricid snail, *Nucella lapillus*, over an 18 d culture period. Likewise, other studies have also failed to raise early-stage excapsulated embryos of this (Stöckmann-Bosbach, 1988) and other prosobranch gastropods (Rivest, 1986). In contrast, Lord (1986) was able to rear 35 pre-shelled embryos of *N. lapillus* over a 21 d period by culturing them within filtered, autoclaved, antibiotic-treated seawater, however, this experiment lasted for only a portion of the developmental period of this species. Although survival rates are undoubtedly higher for later-staged embryos removed from their capsules (Pechenik et al., 1984), embryonic mortality is not the only concern associated with culturing embryos outside their capsule chamber. For those embryos requiring extraembryonic nutrition during development, an artificial culture medium may not always provide embryos with the same accessibility to or the same concentration of nourishment. Consequently, the development of excapsulated embryos may not accurately reflect the normal encapsulated development of muricid gastropods.

Development of embryos within stripped capsules is clearly a useful alternative to raising embryos outside their capsules. Embryos can be viewed through the inner capsule wall during development and still remain enclosed with albumen and nurse eggs in their natural fluid medium. Nevertheless, the high susceptibility of embryos within stripped capsules to contamination by bacteria and protists is still a cause for concern when using this technique. In the present study, embryonic mortality was directly related to the length of time that embryos were cultured within stripped capsules (Fig. 6-4), but differed little among the two culture treatments (Table 6-1). Contamination by protists thus may never be avoided with certainty, especially given that the source of the contamination might be the capsule wall itself. The probability of contamination of stripped capsules by protists can be reduced, however, by studying species with shorter development times than *Nucella emarginata*. Alternatively, if only one particular developmental stage is of interest, capsules could be stripped immediately prior to that stage. Clearly, therefore, the mortality of embryos within stripped capsules can be minimized without reducing this technique's effectiveness.

Despite the high mortality of embryos enclosed within stripped capsules, embryos successfully developed through to hatching within 40 stripped egg capsules over a developmental period lasting as long as 77 days. Thus, for those species of muricid gastropods depositing embryos within opaque capsular cases, such as all five northeastern Pacific species of *Nucella* (e.g. *N. emarginata* [northern and southern species; see Palmer et al., 1990], *N. canaliculata*, *N. lamellosa*, and *N. lima*), this technique provides an effective means of viewing changes in the behavior and morphology of embryos during their intracapsular development. Given the structural similarity between the capsule walls of muricid gastropods and other neogastropod groups (e.g. the Buccinacea; D'Asaro, 1988), this technique may also be applied to other gastropod taxa enclosing developing embryos within multi-laminated capsules.

Stripped egg capsules can also be used to address questions concerning the benefits and costs associated with the deposition of eggs within tough, thick-walled egg capsules. Chaffee & Strathmann (1984) have suggested that the rate of development of embryos enclosed within benthic egg masses may be constrained by the diffusion of oxygen into or metabolic wastes out of these structures. Recently, I have been able to test this by comparing the developmental rate of embryos enclosed within whole versus stripped capsules. My preliminary results have indicated that embryos in stripped capsules do indeed develop significantly faster than embryos in whole capsules (Rawlings, in prep.; Chapter 7), thus suggesting that there is a substantial developmental cost associated with the deposition of embryos within thick-walled capsules.

I have also used stripped egg capsules to examine some of the benefits associated with the encapsulation of eggs. Gastropod egg capsules are generally assumed to be protective, but surprisingly few studies have attempted to determine what exactly capsules protect embryos from (see review in Pechenik, 1986). I have recently examined the effectiveness of the capsule wall in resisting predators by comparing the resistance of whole and stripped capsules to intertidal isopods (Rawlings, 1994). The low survival of embryos within stripped capsules in the present study is also a testament of the ability of the outer capsule wall to resist attack by protists that would otherwise consume these embryos. Stripped egg capsules may also help in furthering our understanding of the role of the capsule wall in acting as an osmotic barrier to solute molecules, and as a filter for the penetrating rays of ultraviolet light (Chapter 5). This technique thus has the potential, under controlled conditions, to be an invaluable tool in understanding more about the development of encapsulated embryos, and the benefits and costs associated with the deposition of eggs within thick-walled capsules.

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Table 6-1. Percentage of stripped and whole capsules in which embryos successfully completed development when cultured under two different conditions (autoclaved, antibiotic-treated seawater, and UV sterilized seawater) in two experiments conducted on different dates. N, number of capsule pairs used in each experiment.

Culture Conditions	Experiment #	% Successful Development:		N	Date Conducted
		Stripped	Whole		
Autoclaved, Antibiotic Treated Seawater	1	100	100	9	September, 1992
	2	35	100	23	July, 1993
	Overall:	53	100	32	
UV Sterilized Seawater	1	50	100	18	September, 1992
	2	38	100	40	September, 1993
	Overall:	40	100	58	

Figure 6-1. Light micrographs of transverse sections through the multilaminated capsule walls of two muricid snails: (A) *Nucella emarginata* and (B) *N. lamellosa*. Symbols L_1 - L_3 refer to the three laminae visible in the capsule wall of these species using light microscopy. Also identified are the two major regions of the capsule wall: the outer capsule wall (OW) comprising laminae L_1 and L_2, and the inner capsule wall (IW) consisting of the innermost lamina, L_3. The opaque outer wall was the portion of the capsule removed during the stripping process. The scale bars represent 50μm.

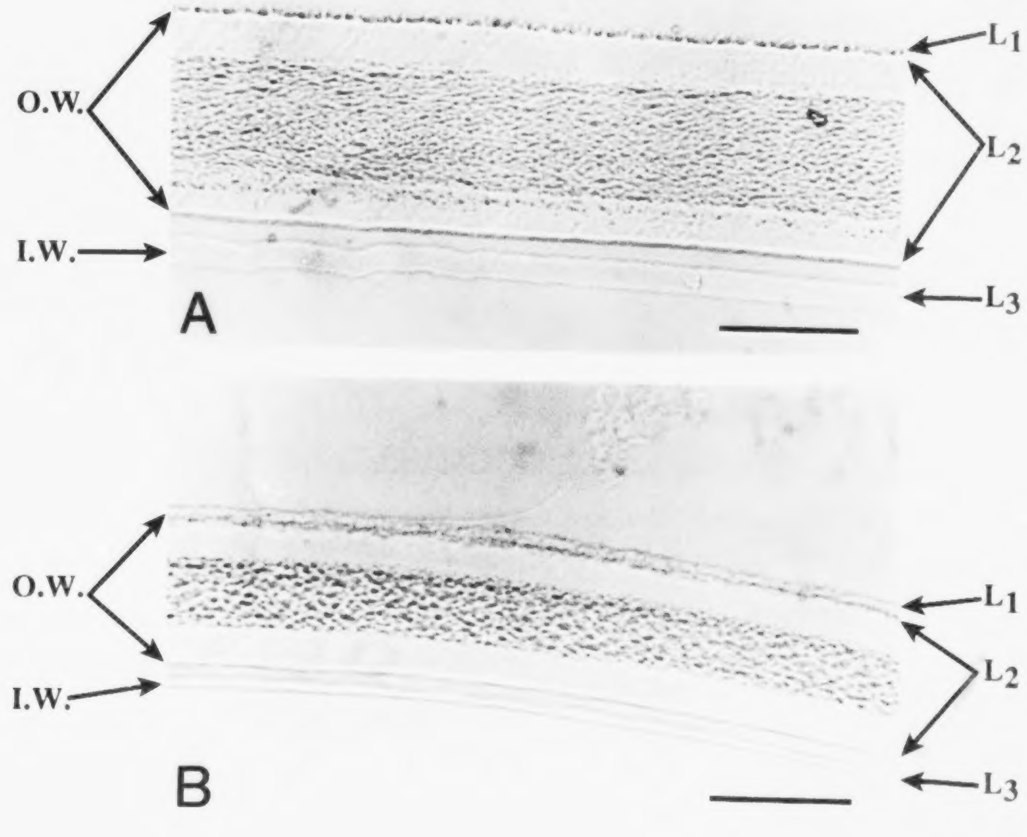

O.W.
I.W.
L1
L2
L3
A
O.W.
I.W.
L1
L2
L3
B

Figure 6-2. Side view (A) and top view (B) of stripped and whole capsules positioned within their Tygon™ holders in the UV sterilized seawater treatment.

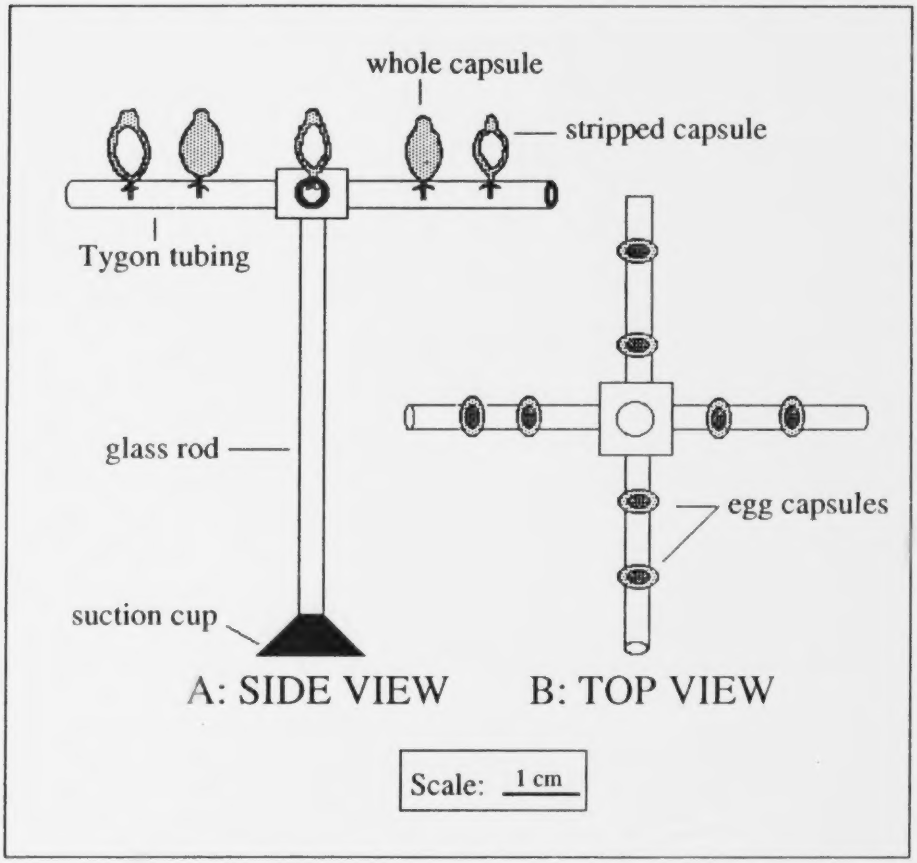

whole capsule
stripped capsule
Tygon tubing
glass rod
suction cup
egg capsules
A: SIDE VIEW
B: TOP VIEW
Scale: 1 cm

Figure 6-3. Developmental sequence of encapsulated embryos of *Nucella emarginata* enclosed within stripped egg capsules. Development times refer to the number of days since capsules were collected from a spawning female. Descriptions of each photographic plate are as follows. (A) Stripped capsule 7 days after collection, illustrating the viscous nature of the capsular fluid and difficulty in distinguishing between nurse eggs and developing embryos at this developmental stage. (B) The same capsule as in (A), 36 days after collection. Note the change in the consistency of the capsular fluid, suggesting the uptake of albumen during development by embryos, and the presence of ciliated late 2nd-stage / early 3rd-stage veligers (see LeBoeuf, 1971, for a description of veliger stages of *N. emarginata*). (C) Thirty-six day old 3rd-stage veligers readily consuming nurse eggs within the capsule chamber. This capsule was collected from the same clutch as the capsule in (A) and (B), indicating differences in development rate of embryos among capsules, possibly due in part to differences in the actual spawning date of capsules within a clutch. (D) The same capsule as in (C) at 60 days of development, illustrating early 4th-stage veligers with an excess of unconsumed nurse eggs. E) Fourth-stage veligers at 60 days of development, revealing the close packing of embryos within the chamber and the complete consumption of all nurse eggs. (F) Newly metamorphosed juveniles within a completely stripped capsule. This capsule was stripped three days prior to the hatching of embryos from the capsule. Scale bars represent 1mm in all photographs.

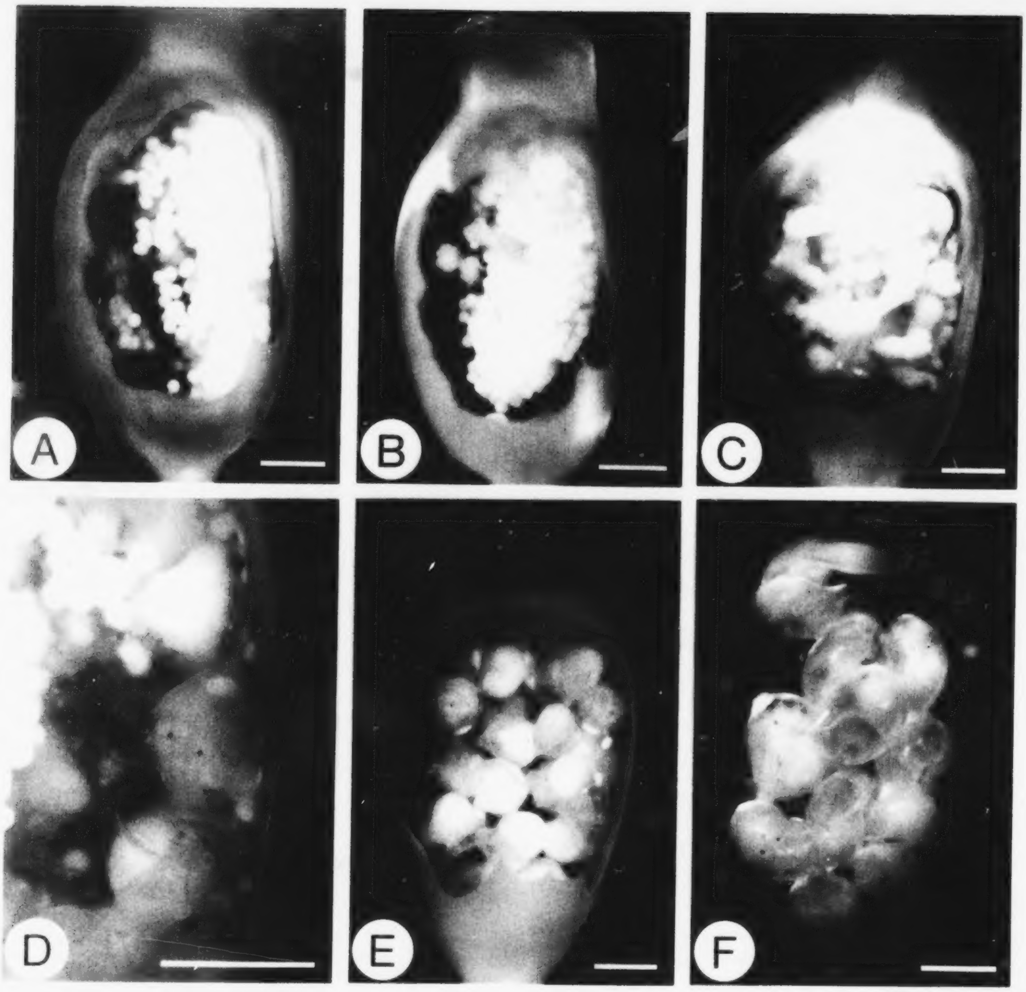
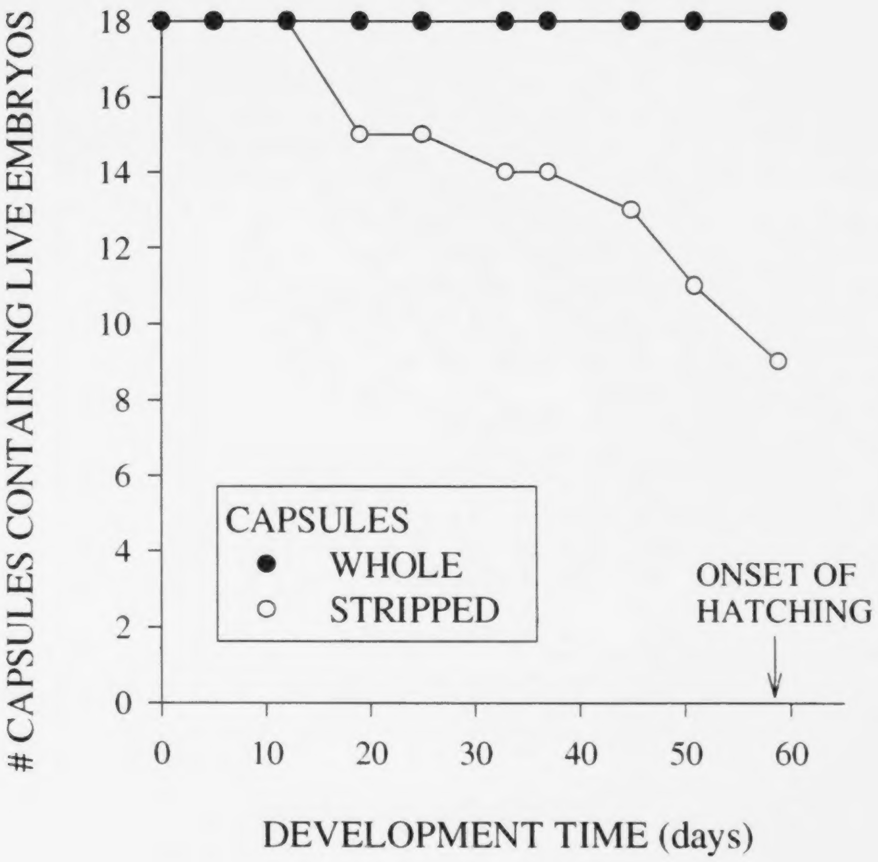

A
B
C
D
E
F

Figure 6-4. Number of stripped and whole capsules containing live embryos when cultured in UV sterilized seawater at 10-12°C from September - October, 1992.



CHAPTER 7

Costs of encapsulation: Diffusive constraints associated with the deposition of embryos within thick-walled capsules

Abstract

Little is known about diffusive constraints associated with the encapsulation of eggs within the tough, fluid-filled capsules of higher prosobranch gastropods. An increase in capsule wall thickness may provide developing embryos with better protection from potentially lethal environmental stresses, but may also limit the exchange of oxygen, nutrients, and metabolic wastes between embryos and their external environment. In the present study, I examined diffusive constraints associated with the deposition of eggs within benthic egg capsules of the rocky shore marine gastropod *Nucella emarginata*. Because the thickness of capsule walls varies significantly among populations of this species, I compared the allocation of embryos within capsules of differing wall thickness. I also examined the development time of embryos within whole and artificially stripped egg capsules, and the influence of environmental conditions, including water motion and aerial exposure, on the rate of embryonic development.

The sturdy, multi-laminated, capsular cases of *Nucella emarginata* do appear to have associated costs. Embryos enclosed within thick-walled capsules take significantly longer to develop and have lower metabolic rates compared to siblings within capsules stripped of their outer capsule wall. Associations between mean capsule wall thickness and the packaging of embryos within capsules collected from different intertidal populations of *N. emarginata* further suggest that capsule walls may place constraints on the number of embryos that can develop successfully within the capsule chamber. In addition, environmental conditions, such as water motion and aerial exposure, can also have a profound effect on the developmental rate of encapsulated embryos.

Although quantitative estimates of the boundary layer and capsule wall resistance to oxygen transfer were not determined, the substantial effects of wall thickness and water motion on embryonic development suggests that these resistances may have an important influence on the packaging and development of embryos within *N. emarginata* egg capsules. Direct measures of the resistance of capsule walls and the boundary layer to oxygen transfer are now necessary to model oxygen transport across the walls of benthic egg capsules under natural field conditions. The outcome of such models should be instrumental in understanding the potential consequences of placing embryos within the

variety of encapsulating structures produced by marine invertebrates.

Introduction

To understand the adaptive significance of enclosing embryos within the diverse array of egg capsules and gelatinous egg masses produced by marine invertebrates, one has to assess both the benefits and costs associated with the deposition of eggs within these structures. Although these elaborate egg coverings may act to protect embryos from a variety of environmental risks during their development (see review in Pechenik, 1986), and to enclose embryos with nutritive reserves such as albumen and nurse eggs (e.g., Clark and Goetzfried, 1978; Spight, 1976a, b; Rivest, 1983, 1986; Rivest and Strathmann, 1994), these same structures may also limit the diffusive exchange of oxygen, nutrients, and metabolic waste products between developing embryos and their external surroundings (Chaffee and Strathmann, 1984; Strathmann and Chaffee, 1984). Hence, variation in the size, shape, thickness, and composition of egg coverings may place constraints on the number, size, and metabolic rate of embryos developing within these structures. By understanding these constraints, therefore, we can then assess the potential consequences of placing embryos within different types of capsules or egg masses, and the effect of these physical limitations on the life-histories of marine invertebrates.

Simple diffusive constraints can have profound consequences for the development of embryos within encapsulating structures. Developing embryos within gelatinous egg masses or thick-walled capsules resemble an equivalent mass of tissue without a circulatory system (see Chaffee and Strathmann, 1984). Oxygen transport to these embryos occurs by diffusion across the unstirred jelly or capsule wall, involving processes driven by differences in concentration or chemical potential, rather than by total or hydraulic pressure differences associated with convective flow. Although the diffusion coefficients of egg mass jelly and capsule walls have been difficult to measure (but see Burggren, 1985; Cohen and Strathmann, submitted), both anecdotal and experimental evidence suggest that thick, gelatinous egg coverings can limit the rate of embryonic development. In aquatic habitats, for instance, retarded development or even death has been reported in centrally-positioned embryos within the egg masses of amphibians (e.g., Dempster, 1933; Grainger, 1959; Salthe and Mecham, 1974, but see Burggren, 1985), polychaetes (Chaffee and Strathmann, 1984), and opisthobranch and pulmonate gastropods (Borland, 1950; Chaffee and Strathmann, 1984; Marois and Croll, 1991, Strathmann and Strathmann, 1995). Likewise, centrally-deposited embryos within some species hatch at smaller sizes than embryos located at the periphery of these masses (Strathmann and Strathmann, 1995). These effects appear to result primarily from the limited availability of oxygen at the centre of these egg masses, rather than the accumulation of metabolic wastes, since: 1) developing embryos have a high tolerance of metabolic waste products (e.g., fish embryos: Giorgi and Congleton, 1984; gastropod embryos: Strathmann and Strathmann, 1995), 2) oxygen concentrations are often low within the centre of gelatinous egg masses (Seymour and Roberts, 1991; Pinder and Friet, 1994; Cohen and Strathmann, submitted), and 3) embryos develop synchronously within thick egg masses when cultured in seawater supersaturated with oxygen (Strathmann and Strathmann, 1995). Based on observations of asynchronous development within a variety of naturally deposited egg masses, therefore, many egg masses may approach or even exceed the diffusive limits necessary for the successful development of embryos embedded within (Cohen and Strathmann, submitted; Lee and Strathmann, in prep.).

Constraints associated with the packaging of eggs within benthic egg capsules or gelatinous egg masses can differ substantially based on the size, shape, and thickness of the egg mass (Chaffee and Strathmann, 1984; Strathmann and Strathmann, 1989; 1995). Using simple diffusion models, for instance, Strathmann and Chaffee (1984) showed that gelatinous masses and fluid-filled capsules should impose different physical limitations on embryonic development. Differences in the shape of egg masses can also affect these constraints. Embryos embedded within thin gelatinous ribbons with large surface area to volume ratios, for instance, do not exhibit asynchronous development evident in thicker, globose masses of other species, despite the increased density of embryos packaged within thin egg ribbons (Chaffee and Strathmann, 1984; Strathmann and Strathmann, 1989; Lee and Strathmann, in prep.). Hence, diffusive constraints associated with one type or shape of egg mass cannot necessarily be considered true for other egg coverings.

Constraints on embryonic development may also vary substantially depending on the habitat in which egg masses are deposited (Chaffee and Strathmann, 1984; Strathmann, 1990). In aquatic environments, constraints associated with the size and thickness of egg coverings may be more severe because of the lower diffusivity and solubility of oxygen and carbon dioxide in salt and fresh water versus air (Prosser and Brown, 1961). Also, based on the higher viscosity of water, and lower velocities of water movement relative to air, oxygen deficient boundary layers surrounding egg masses are likely to be thicker in water (Vogel, 1981), thereby slowing the exchange of oxygen across the egg mass. Even within marine environments, constraints may differ substantially among habitats. Changes in oxygen solubility associated with water temperature differences, for instance, have been suggested to explain geographic differences in the type of brooding / encapsulation patterns exhibited by some marine organisms (e.g., opercular brooding in spirorbid polychaetes; see Hess, 1993). Water movement can also affect boundary layer conditions surrounding egg masses. The deposition of egg masses within water currents can substantially increase the speed of embryonic development by reducing the thickness of the oxygen deplete boundary layer (Chaffee and Strathmann, 1984; Strathmann and Strathmann, 1990, and references therein). Eggs within capsules or masses completely immersed, or exposed to fluctuating cycles of immersion and emersion, may thus experience different constraints than those developing in an aerial environment. To understand the limitations imposed upon embryos by their egg coverings, therefore, one must also consider the specific environmental conditions under which these egg masses are deposited.

Although a number of studies have examined the effects of egg mass size, shape and thickness on the diffusion of oxygen through the gelatinous egg masses of amphibians (Burggren, 1985; Seymour and Bradford, 1987; Seymour and Roberts, 1991; Pinder and Friet, 1994), polychaetes (Chaffee and Strathmann, 1984; Cohen and Strathmann, submitted), opisthobranch and pulmonate gastropods (Chaffee and Strathmann, 1984; Marois and Croll, 1991; Strathmann and Strathmann, 1989; 1995; Cohen and Strathmann, submitted) and crustaceans (Crisp, 1959), little is known about the diffusion of oxygen through the tough, thick-walled egg capsules of higher prosobranch gastropods, such as those produced by neogastropods. Strathmann and Chaffee (1984) reasoned that fluid-filled egg capsules should not limit the exchange of materials between embryos and their environment as severely as other types of egg masses, assuming that the fluid within these capsule is continually mixed by the developing embryos. Based on their model, the number of embryos within spherical, fluid-filled capsules should increase in proportion to the surface area (i.e., radius squared) of the capsule, and decrease in proportion to the thickness of the capsule wall, as follows:

$$N \cdot M = \Delta C \cdot A \cdot D \cdot / T$$

where N = number of embryos, M = metabolic rate per embryo (µl/s), ΔC = concentration gradient of oxygen across the capsule wall (µl/m^3), A = capsule surface area (m^2), D = diffusion coefficient for capsule wall material (m^2/s) and T = capsule wall thickness (m). In this model, the supply of oxygen is limited only by the rate of diffusion through the capsule wall, and the oxygen supply is in equilibrium with consumption by developing embryos.

Under natural conditions, oxygen molecules must also pass through a boundary layer prior to diffusing across the capsule wall and entering the well-mixed capsular chamber. If the boundary layer and capsule wall are considered as two resistances in series, then the larger of these resistances will be the most limiting to oxygen transport. These two resistances can be written as:

$$N \cdot M = (A \cdot \Delta C)/(r_b + r_w) = \Delta C/[\ (1/(A \cdot\ h_m)) + (T/(A \cdot D))]$$

where $r_b + r_w$ are the resistances of the boundary layer and capsule wall, respectively (s/m), and h_m is the mass transfer coefficient (m/s), describing the ease of movement of oxygen across the boundary layer (see White, 1988). Clearly, therefore, if r_b is large relative to r_w then environmental parameters affecting boundary layer conditions, such as the velocity and viscosity of the medium in which capsules are deposited, will also place substantial constraints on embryonic development. Conversely, if r_w is high relative to r_b, then these environmental parameters can be largely disregarded. To understand the importance of diffusive constraints associated with the capsule wall, therefore, the relative magnitude of these two resistance terms must be estimated.

In the present study I examined diffusive constraints associated with the deposition of eggs within the tough, fluid-filled capsules of the rocky shore gastropod, *Nucella emarginata* (northern species; Palmer et al., 1990). Because the capsules walls of *Nucella emarginata* are known to vary significantly in wall thickness among populations of this species (Rawlings, 1990, 1994; Chapter 2), and because wall thickness has been suggested to limit the exchange of materials between embryos and their surroundings (Strathmann and Chaffee, 1984; Perron and Corpuz, 1982; Etter, 1989), I compared the allocation of embryos among capsules of differing wall thickness. I also examined the effect of wall thickness on the developmental time of encapsulated embryos, as well as the influence of water motion and aerial exposure on embryonic development.

Materials and Methods

Field collection of *N. emarginata* capsules

To examine the packaging of embryos within capsules of *Nucella emarginata*, I collected ten clutches of capsules from each of 10 different populations in Barkley Sound between June - July, 1991. At each site, the intertidal zone was scoured for clutches of egg capsules and the first ten clutches containing early-stage embryos were collected.

These capsules could usually be identified by the appearance of large numbers of nurse eggs through the capsule wall, and the lack of well-developed shelled embryos. The collection of capsules containing young embryos minimized the potential for variation in the number of embryos per capsule to result from site-differences in embryonic mortality during development. Once in the laboratory, five capsules were selected from each clutch and then individually allocated to their own site- and clutch-specific mesh-paneled (600 μm Nitex) microfuge cages (see Chapter 3, for a diagram of these cages). These cages allowed sufficient water circulation for encapsulated development to occur normally, but also acted to retain even the smallest juvenile snails once they hatched from their capsules. Cages were strung from a monofilament line and then suspended in a tank of aerated, flowing seawater. Seawater temperatures varied from 10 to 12°C over the course of development. Vials were examined at weekly intervals, at which time the mesh-panels of the microfuge cages were scrubbed to avoid fouling by diatoms. As embryonic development neared completion, cages were checked every two days for the presence of hatchlings. Upon hatching from their capsules, juveniles were counted and preserved in 70% alcohol. At this time, I also measured the body length (excluding the stalk), plug length, and the width and length of the capsule chamber (seam and side view), using a dissecting microscope (6 - 12 x) equipped with an ocular micrometer. The volume of the chamber was determined using the formula for a prolate ellipsoid: $V = 4/3\pi(a/2)(b/2)^2$, where a and b are the average chamber lengths and widths, respectively.

A second group of ten clutches of freshly-deposited *Nucella emarginata* capsules was also collected from each site in the summer of 1991. These capsules were used for measurements of capsule wall thickness. Three capsules were collected from each clutch and then measured under a dissecting microscope at 6 - 12 x magnification. Capsules were marked at a point 70% along the length of the chamber housing developing embryos, and then sectioned at the marked point using a freeze microtome. Capsule walls are thinnest and least variable in this region (see Rawlings, 1990). Capsule sections were then mounted under a compound microscope equipped with an ocular micrometer. Wall thickness was determined by taking the average of eight measurements recorded around the circumference of the mounted section, using the two seams of the capsule wall as reference points.

Development rates of embryos within stripped and whole capsules

Because differences in capsule wall thickness among populations of *Nucella emarginata* were confounded by differences in capsule shape and size, I opted to manipulate capsule wall thickness experimentally to examine its effect on the development rate of embryos. To do this, I compared the development times of embryos enclosed within unmanipulated capsules versus those within capsules that had been stripped of a large portion of the outer capsule wall. The methods used in this experiment have been described in detail elsewhere (Chapter 6; Rawlings, 1995). Briefly, capsules were obtained from a group of snails born and raised entirely within the laboratory. To collect capsules, cages containing individual female *N. emarginata* were checked every one to two weeks for the presence of new capsules. When fresh capsules had been spawned, two adjacent capsules were collected from each clutch. Because the exact spawning date of each capsule was rarely known, this protocol increased the likelihood that the paired capsules were spawned at a similar time. Once capsules were collected, both were swabbed with 70% alcohol to sterilize their outer surfaces. One capsule was then stripped of approximately two-thirds of its outer wall using a Staedler Histostat microtome blade, while the remaining capsule was left intact (hereafter termed "whole"). Capsules were carefully placed in Tygon holders (see Chapter 6; Rawlings, 1995) that had been sterilized in 70% alcohol and air-dried prior to being used. Tygon holders were positioned in plastic 10 L culture chambers and provided with a steady flow (2 L/min) of 1 µm filtered UV sterilized seawater (10 - 12°C). At approximately five day intervals, capsules were checked for signs of mortality; and gently swabbed with 70% alcohol to remove any surface debris. Tygon holders were also changed at this time, and replaced with freshly sterilized holders. Once embryos reached the late 4th veliger stage (LeBoeuf, 1971), as observed through the transparent inner walls of stripped capsules, both stripped and whole capsules were checked daily for any evidence of hatching snails.

This experiment was conducted for a total of eight pairs of capsules in the fall of 1992 and 15 pairs of capsules in 1993. The actual number of capsules used in these experiments was much higher, but embryos within many stripped capsules died due to contamination by a flagellated protist (see Chapter 6; Rawlings, 1995). In 1992, juvenile snails were scored as "hatching" when the first snail emerged through the capsular aperture. However, because juvenile snails occasionally became stuck in the plug region, thus preventing the escape of hatchlings from the capsule, I changed my procedure slightly in 1993. In this experiment, juveniles were recorded as hatching as soon as the plug had dissolved. This was determined by gently prodding the plug of capsules containing late-stage embryos at daily intervals using a blunt probe. Once a plug had dissolved, hatchlings were removed and counted. If juveniles within only one capsule of a pair hatched, the other capsule was checked daily for evidence of hatching snails. To ensure that all hatchlings within an open capsule had actually metamorphosed, all were examined under a dissecting microscope (25 - 50 x) for the presence or absence of a velum. As both experiments were conducted in identical fashion, data were pooled for final comparisons of development times between pairs of stripped and whole capsules.

Metabolic rates of embryos within stripped and whole capsules

The metabolic rates of embryos enclosed within whole and stripped capsules were measured to determine if the presence of the outer capsule wall limited the rate of oxygen uptake. Experiments were conducted in an experimental chamber consisting of a 10 ml glass vial (Fig. 7-1; inner diam. = 19 mm; length = 50 mm), with a capsule stand (inner diam = 15 mm; length = 15 mm) mounted within to secure capsules during the course of an experiment. A small stir bar (flea) was placed in the bottom of the vial to keep water in the chamber circulating, and the chamber was positioned within a water-cooled jacket (12 °C) on top of a magnetic stirrer. A Radiometer oxygen probe was also mounted within the chamber to monitor the oxygen content of seawater during the experiment. The experimental chamber was filled with filtered (1 µm), UV-sterilized seawater and then sealed by covering the surface meniscus of seawater with a thick layer of mineral oil. PO_2 levels were measured using a Radiometer PHM71MK2 Acid Base Analyzer and data were recorded at 10 minute intervals using a data acquisition system. In all trials, water used within the chamber had been placed within a water bath (12°C) and bubbled with ambient air for at least 30 minutes prior to conducting the experiment. The probe was recalibrated before each trial by bubbling either nitrogen or air through two calibration flasks of seawater within a water bath at 12°C.

Each experimental replicate consisted of three trials. Initially a control trial was conducted to measure the consumption of oxygen by the probe and any background consumption of oxygen within the chamber. To do this, PO_2 levels were recorded for sealed chambers over a 16 to 24 h period (control treatment). A second trial was then conducted in which five laboratory-laid *Nucella emarginata* capsules containing late 4th-stage veligers (see LeBoeuf, 1971) were placed within the chamber (whole capsule treatment). Prior to using these capsules, the exterior of each capsule was gently swabbed with 70% alcohol to sterilize the outer surface, and capsules were then left overnight in sterilized seawater (12°C). Capsules were positioned within the chamber by poking their stalks through the mesh top of the capsule stand. The vial was then flooded with seawater, bubbles carefully removed, and the chamber sealed with mineral oil. This experiment usually lasted from 16 to 24 h, or until PO_2 levels dropped below 40 Torr within the experimental chamber. The final experimental trial consisted of repeating the above procedure, but using capsules that had been stripped of their outer wall (stripped capsule treatment). Capsules used in the previous trial were swabbed with 70% alcohol to sterilize the outer surface and were then gradually peeled to remove the outer capsule wall (hereafter termed "stripped"), as described previously. Once capsules had been stripped, they were left for 12 h in sterilized seawater (12°C) prior to testing. Capsules were then mounted in the chamber as described above.

This experimental sequence was repeated five times with five different groups of capsules. Prior to conducting each trial, the volume of seawater used in each trial was determined by weighing the filled chamber, subtracting the weight of the glass vial, and then correcting for the salinity of seawater (1.025 g/ml at 32‰). Following each experiment, the volume of each capsule was determined by water displacement. This allowed the total volume of the chamber to be estimated (i.e., water plus capsule volume), and thus, the amount of oxygen within the chamber to be known at any point during the experiment. The dry weight of embryos was also measured. Embryos were removed from their capsules, counted, and then frozen. At a later date, embryos were thawed and soaked in EDTA to dissolve away their shells. As soon as their shells had completely dissolved, embryos were rinsed in distilled water, and then dried en masse at 60°C.

Effect of environmental conditions on development rates of encapsulated embryos of *Nucella emarginata*

a) Immersion versus emersion

If developmental rates of encapsulated embryos are limited by diffusion-dependent processes, then development time may also reflect a) the frequency of exposure of capsules to air versus water, because of the different physical properties of these media, and b) the movement of the fluid medium in which capsules are developing. To examine the effect of aerial exposure on the developmental rate of encapsulated embryos, I constructed an experimental apparatus to manipulate the duration of time that capsules spent exposed to air. This set-up was designed to produce two experimental conditions based on a 12 h cycle: 1) continuous immersion, and 2) an immersion / emersion cycle (6 / 12 h immersed, 6 / 12 h emersed). The experimental apparatus (see Fig. 7-2a) consisted of a water pump, a header tank, two culture chambers, a collecting tank, and a timing device. At six hour intervals, a timing device either activated or deactivated a water pump. Once activated, water was continuously pumped from a 15 L collecting tank to a 12 L header tank, from there draining down into two 3 L culture chambers (1.2 L/min in each chamber) and overflowing into the collecting tank below. Water was continuously recirculated in this system until the pump was deactivated six hours later. Once the pump was turned off, water drained completely from the header tank into the collecting tank below. Because of a small hole (3 mm diameter) drilled at a height of 60 mm from the bottom of each culture chamber (Fig. 7-2b), water drained from the top half of each culture chamber, leaving the lower part of the chamber full of water. Once the pump was reactivated, water was pumped back into the header tank and flowed down into the culture chambers below. Because the flow rate of water from the header tank into the culture chamber was substantially higher than the flow rate out of each drain hole, the culture chambers were always full and overflowing when the pump was turned on.

Using this apparatus, I was able to assign capsules to two treatment conditions simply by altering the height at which capsules were positioned within each culture chamber. Capsules suspended above the drain hole were exposed to an immersion / emersion cycle every six hours. Capsules suspended below the drain hole spent the entire 12 h immersed: 6 h in fast moving seawater and 6 h in still seawater. To ensure that the temperature of the air and seawater did not differ, this experiment was conducted in an incubator at 12°C.

Capsules were mounted on two-tiered Tygon holders (Fig. 7-2b). In this way, capsules could be suspended at two heights within the culture chamber. Paired capsules were collected from isolated female snails in the laboratory, as described previously, and one capsule from each pair was randomly allocated to each treatment condition (complete immersion *vs.* immersion / emersion). Autoclaved, antibiotic-treated (0.050 g/L streptomycin; 0.030 g/L penicillin) seawater was changed at approximately 5 d intervals, as were capsule holders. Paired stripped capsules were also assigned to both treatment conditions, however, embryos within these capsules did not survive due to contamination by protists within the recirculating system.

b) Moving versus still water

I examined the effects of water motion on development rate of *Nucella emarginata* capsules to determine the importance of boundary layer effects on embryonic development. This was done by rearing capsules in 3 L glass culture jars containing either still or moving seawater. Capsules containing up to two-week old embryos were collected in pairs from isolated laboratory-reared female snails, as described previously, and were then separated; one capsule was placed in a Tygon holder allocated to the moving water treatment, while the second capsule was placed in an identical holder allocated to the still water treatment. A stirring system consisting of Plexiglas paddles (6 rotations/min) and a rotisserie motor (see Strathmann, 1987) served to keep water within the "moving" seawater treatment continuously stirred during this experiment. Culture jars containing capsules allocated to the two different treatment conditions were placed side by side in a glass aquarium tank filled with seawater. Seawater trickled though this tank and served to keep both culture jars at the same temperature. This set-up was replicated twice. Autoclaved seawater was changed at 4 to 6 d intervals, as were the glass culture jars. Capsules were checked at regular intervals for signs of hatching snails. As soon as embryos reached the late 4th-veliger stage, capsules were checked daily. Water temperature varied from 10 - 11 °C during the course of this experiment.

Results

Field collection of *N. emarginata* capsules

Egg capsules of *Nucella emarginata* differed substantially in size and shape among the ten populations sampled in Barkley Sound (Fig. 7-3a, b, c, d). Capsules from the most wave-sheltered site, Grappler Inlet, were the largest in overall size, but had proportionately smaller chamber widths per unit chamber length compared to capsules from other sites. In contrast, capsules from most wave-exposed shores were generally smaller in size, but stockier in appearance, with considerably larger chamber widths per unit chamber length than capsules from more wave-sheltered locales. Differences in capsule size and shape resulted in marked variation in the volume of capsule chambers among populations (Fig 7-3d).

The number of hatchling snails enclosed within each capsule varied substantially both within and among populations (Fig 7-3e). Within each population, variation in capsule chamber volume explained up to 64.4 % of the variation in the total number of hatchlings packaged per capsule (Table 7-1), however, relationships between hatchling number and capsule volume were significant for only five out of ten sites. For those relationships that were significant, scaling coefficients (RMA slopes) varied from 0.87 - 1.78, and did not differ significantly from a slope of 1.0 (see methods for among slope comparisons of RMA regressions in McArdle, 1988). Hence, the number of hatchlings per capsule appeared to scale isometrically with capsule volume.

Among populations, the number of embryos packaged per capsule varied almost two-fold, with as few as 11.5 ± 0.89 hatchlings allocated per capsule at Voss Point (mean ± SE; n = 10 clutches) and as many as 21.8 ± 2.27 hatchlings per capsule at Grappler Inlet (Fig. 7-3e). When scaled for differences in capsule volume, the mean number of hatchlings per unit capsule volume still varied substantially among sites (Fig. 7-4). Compared to other populations, capsules from both wave-exposed (e.g., FG, CB, KP) and wave-sheltered extremes (GR) had considerably lower numbers of embryos per unit capsule volume. Even though these capsules were, on average, larger than capsules from other sites, comparisons at similar volumes indicated that capsules from wave-exposed and wave-sheltered extremes had substantially lower numbers of embryos than those from more intermediate sites (Fig 7-5).

Because the packaging of embryos within capsules could also reflect constraints associated with the diffusion of materials through capsule walls, I examined the relation between the number of hatchlings per unit volume and the mean wall thickness of capsules collected from each site in 1991. The wall thickness of capsules varied substantially among sites (Fig. 7-6 inset), with thicker-walled capsules present along both wave-sheltered and wave-exposed shores. The number of hatchlings per unit capsule volume was inversely related to the wall thickness of the capsular chamber (Fig. 7-6), with 58.2% of the variation in embryo/volume ratio accounted for by site-differences in capsule wall thickness.

Development rates of embryos within stripped and whole capsules

Embryos within stripped capsules developed 10.1% and 13.9% faster than embryos in whole capsules in two separate experiments conducted in 1992 and 1993 (Fig. 7-7). Mean (± SE) development time was 61.3 ± 0.52 and 68.2 ± 0.71 days (n = 7) for embryos within stripped and whole capsules in 1992 (at 10 - 12°C), and 58.7 ± 1.60 and 68.1 ± 2.07 days (n = 15) for embryos within stripped and whole capsules in 1993 (at 10 - 12°C). Embryos in only one of 23 whole capsules hatched before their siblings in stripped capsules (Fig. 7-7); delayed hatching in this one stripped capsule appeared to be associated with the occlusion of the capsule aperture by a juvenile snail. Because embryos within capsules may have been up to two weeks old prior to allocating them to treatment conditions, differences in the development time of embryos between stripped and whole capsules could have been even more substantial if compared over their full embryonic period.

The number of hatchlings emerging per capsule was compared among pairs of capsules in 1993 to ensure that differences in development time did not reflect differences in the packaging of embryos per capsule. No significant differences were evident in the number of hatchlings within whole versus stripped capsules (mean # hatchlings ± SE: 10.4 ± 1.66 and 12.3 ± 1.80 for whole vs. stripped capsules, respectively; paired t-test: t = 1.52, P = 0.15; n = 15 pairs). Development of embryos in both stripped and whole capsules appeared to proceed normally. Although a small percentage of embryos within these capsules had not metamorphosed at the time of plug dissolution (3.1% of 160 embryos within whole capsules and 6.0% of 183 embryos within stripped capsules), this slight asynchrony in the development of capsulemates is not uncommon even in field-collected capsules of *Nucella emarginata* (Spight, 1981; Rawlings, pers. obs.).

Metabolic rates of embryos within stripped and whole capsules

Although there was a small, linear uptake of oxygen in the experimental chamber during control trials, this represented, on average, only 4.6% of the oxygen consumed by embryos during experimental trials. This background consumption of oxygen was subtracted from rates of consumption during experimental trials to estimate the actual metabolic rates of developing embryos with whole and stripped capsules.

PO_2 levels declined exponentially over time when capsules were placed within the sealed respirometer, thus indicating that the rate of oxygen consumption by encapsulated embryos was dependent on external oxygen concentrations. Because of this, I compared oxygen consumption rates among embryos within whole and stripped capsules at 20 Torr intervals, ranging over external oxygen pressures from 140 - 40 Torr. The amount of oxygen consumed per embryo (μl/s) differed significantly between capsule treatments (Fig. 7-8): metabolic rates of embryos were significantly higher in stripped capsules than within whole capsules over the range of PO_2 values examined. Rates of oxygen consumption per embryo were, on average, 21.3 % and 28.8 % higher within stripped versus whole capsules at high (140 - 120 Torr) and low (60 - 40 Torr) PO_2 concentrations, respectively.

Oxygen consumption of veligers within stripped egg capsules ranged from 8.29 - 13.83 x 10^{-6} μl/s of O_2 (120 - 140 Torr) for average dry weights of hatchlings of 113.9 - 184.8 μg. These metabolic rates were within the range of published values for molluscan veligers, given the dry weight of these embryos, and the temperature (12°C) at which these experiments were conducted (see Pechenik, 1980; Bayne, 1983). The observed change in metabolic rate with decreasing external oxygen concentrations, however, appears unusual for gastropod embryos, at least over the range of partial pressures examined here (see Strathmann and Strathmann, 1995).

Effect of environmental conditions on development rates of encapsulated embryos of *Nucella emarginata*

a) Immersion versus emersion

Embryos exposed to a 6 h immersion / 6 h emersion cycle developed, on average, 8.1% faster than clutchmates assigned to a complete immersion cycle (Fig. 7-9). Development time of embryos were 47.7 ± 1.28 days versus 43.9 ± 1.09 days (n = 26 pairs; @ 12°C) for capsules within complete immersion and immersion/emersion treatments, respectively;

these differences were significant (Paired t-test: t = 5.22, P < 0.001). Only four capsules from a total of 26 capsule pairs contained embryos which developed faster within the complete immersion cycle. The number of embryos within capsules assigned to both treatments were not significantly different, with means (± SE) of 14.1 ± 1.34 embryos and 13.0 ± 1.49 embryos per capsules in the immersion versus the immersion/emersion treatments, respectively.

b) Moving versus still water

Water movement also had a significant effect on the rate of development of *Nucella emarginata* embryos (Fig. 7-10). Embryos within capsules exposed to moving water developed, on average, 20.8 % faster than those within capsules exposed to still water, with mean development times of 75.2 ± 4.37 days and 94.9 ± 5.97 days (n=12 capsule pairs; @ 10 - 11°C) for embryos in the moving versus still water treatments, respectively. These differences in development time were significant among treatment conditions (t test, t = 7.18, P < 0.001, n = 12).

Discussion

Diffusive constraints associated with fluid-filled egg capsules

The deposition of *Nucella emarginata* embryos within sturdy, multi-laminated, capsular cases does appear to have associated costs. Embryos enclosed within thick-walled capsules took significantly longer to develop (Fig. 7-7) and had lower metabolic rates compared to siblings within capsules stripped of their outer capsule wall (Fig. 7-8). Because an increase in development time can increase the probability of exposure of developing embryos to foraging predators or lethal physical stresses, if all other things are equal, embryos with prolonged development will suffer higher stage-specific mortality rates than those with shorter development. Associations between mean capsule wall thickness and the packaging of embryos within capsules collected from different intertidal populations of *N. emarginata* also suggest that capsule walls may place constraints on the number of embryos that can develop successfully within the capsule chamber (Fig. 7-6). The presence of these costs of encapsulation indicates, therefore, that the production of thicker-walled capsules may reflect environmental conditions where the protective benefits of enclosing embryos behind thicker barriers (e.g., Perron, 1981; Perron and Corpuz, 1982; Rawlings, 1990; 1994; Chapter 3, 4, 5) outweigh both the increased diffusive constraints associated with the exchange of materials between embryos and their environment (Strathmann and Chaffee, 1984), and the presumed higher energetic expense of thicker capsule walls (see Perron, 1981; Perron and Corpuz, 1982; Chapter 2).

Based on a simple diffusion model, Strathmann and Chaffee (1984) predicted that an increase in the wall thickness of fluid-filled egg capsules should be matched by a concomitant decrease in either the number of embryos per capsule or the metabolic rate of these embryos. The results of this study provide support for both of these predictions: the developmental rate of embryos was prolonged in whole capsules relative to stripped capsules (Fig. 7-8), and the partitioning of embryos per unit capsule volume declined with an increase in the thickness of the capsule walls among populations of *N. emarginata* (Fig. 7-6, 7-7). Nevertheless, these results should be interpreted with caution. Associations between capsule wall thickness and the number of embryos per unit capsule volume do not necessarily imply causal effects. For instance, this relationship could also reflect a direct tradeoff in the allocation of energy into protective extraembryonic materials versus eggs, such that more energy invested into capsular cases (i.e., thicker walls) results in less energy available for eggs (i.e., fewer nurse eggs and embryos). Differences in the packaging of embryos within capsules can indeed occur in response to proximate changes in food conditions (e.g., Spight and Emlen, 1976; McKillup and Butler, 1979; Chapter 2), however, I found no evidence of a corresponding change in the thickness of capsule walls (see Chapter 2). Alternatively, associations between capsule wall thickness and the packaging of embryos per capsule could result from similar selective pressures acting upon both encapsulated embryos and juvenile snails. The production of thicker-walled capsules among some populations of *Nucella emarginata,* for example, may be a response for the increased protection of developing embryos from local environmental stresses (Rawlings, 1990; 1994). Likewise, the deposition of fewer embryos per unit capsule volume may result in larger sizes of hatchling snails (e.g., Etter, 1989), since this ensures less competition among embryos for food within the capsule chamber (e.g., nurse eggs and/or albumen; Spight, 1976a; Gallardo, 1979; Rivest, 1983), and sufficient space for embryos to grow during development. A larger size at hatching may confer a substantial advantage to temperate rocky shore gastropods (Spight, 1976b; Rivest, 1983; Etter, 1989), including decreased vulnerability to predators (Spight, 1976b; Rivest, 1983; Gosselin, 1994). Hence, the deposition of fewer embryos per capsule within thicker walled capsules may result from common adaptive responses to the increased severity of specific environmental risks at some intertidal sites.

Somewhat surprisingly, the packaging of embryos within *Nucella emarginata* capsules did not appear to be constrained by the amount of respiratory surface provided by the capsule walls. The number of embryos per capsule scaled most closely with capsule volume than any other measure (Table 7-1). In the egg capsules of *Nucella lapillus*, Pechenik et al. (1984) also reported embryo number per capsule to scale with capsule volume, although Etter (1989) found RMA slopes of this geometric relationship to vary between 0.86 and 2.06 depending on the population and year examined. These findings conflict somewhat with other studies of neogastropod egg capsules. For instance, Perron and Corpuz (1982) noted that the number of eggs within the flattened capsules of *Conus pennaceus* scaled directly with capsule surface area. Others have also reported approximately linear relations between the number of embryos per capsule and capsule length (e.g., *Nucella crassilabrum*, Gallardo, 1979), or length squared (*Nucella* spp., Spight and Emlen, 1976), indicating that embryo number per unit capsule volume must decrease with increasing capsule size. Nevertheless, in all these species (except *Conus pennaceus*; Perron and Corpuz, 1982), capsule volume accounted for less than 40% of the variation in embryo number per capsule (Spight and Emlen, 1976; Pechenik et al., 1984, Etter, 1989). Hence, scaling arguments become somewhat moot when there are clearly many other factors affecting the provisioning of embryos to capsule chambers.

Scaling constraints associated with the packing of embryos within *N. emarginata* capsules may be evident when examining the ash free dry weight of embryonic material per capsule rather than number of embryos per capsule. Because *N. emarginata* embryos feed on nurse eggs during development, the size of developing embryos varies considerably within and among capsules based on the number of nurse eggs they have consumed. In capsules with few embryos, embryos have less competition for nurse eggs, and thus grow to larger sizes than embryos with many capsulemates (Spight, 1976a, b; Gallardo, 1979; Rivest, 1983; Etter, 1989). Hence, the weight of metabolizing tissue per capsule is not just a simple function of the number of embryos per capsule, but also the ratio between the number of nurse eggs and embryos. In species of *Nucella* with nurse eggs (e.g., *Nucella lapillus*, *N. crassilabrum*, *N. lima*, and *N. emarginata*; Collins et al., submitted), therefore, embryo volume or the total ash free dry weight of embryonic material per capsule may be a more meaningful variable to use in examining scaling constraints associated with the packaging of eggs within capsules. Work is currently in progress to determine how the dry weight of *Nucella* embryos per capsule scales with chamber volume.

Models of oxygen diffusion through egg masses and capsules

Diffusion models provide the key to assessing the number of embryos that can develop successfully within benthic egg capsules or gelatinous egg masses, given the constraints imposed upon them by the thickness and surface area of the egg coverings, and environmental conditions in which capsules are deposited (e.g., Strathmann and Chaffee, 1984; Cohen and Strathmann, submitted; Lee and Strathmann, in prep.). An essential parameter in these models is the diffusion coefficient of the egg covering to oxygen (Cohen and Strathmann, submitted), since this describes the movement of oxygen through the encapsulating material due to molecular agitation (Monteith and Unsworth, 1990). Despite considerable interest in the diffusive exchange of oxygen across egg mass jelly, however, few attempts have been made to measure the diffusivity of gelatinous egg coverings to oxygen directly. Because of this, some models have assumed the diffusion coefficient to be equivalent to that in water (Crisp, 1959; Strathmann and Chaffee, 1984). Fortunately, recent studies have indicated that this assumption may be reasonable. Burggren (1985) estimated the diffusion coefficient for the egg mass jelly of the pickerel frog, *Rana palustris,* to be 75% of that of oxygen in distilled water at the same temperature and pressure. Likewise, Cohen and Strathmann (submitted) calculated the diffusion coefficient of egg masses of the opisthobranch *Melanochlamys diomeda* to be only slightly lower than that in water. Thus, for gelatinous egg masses at least, the slowed transport rate of oxygen across egg masses is not the consequence of a low diffusion coefficient of egg mass jelly. Instead, the retarded development of centrally-positioned embryos must result from the fact that the thick jelly mass is an unstirred barrier to oxygen transport (Cohen and Strathmann, submitted). Channels through jelly egg masses (Pinder and Friet, 1994) or the spinning of embryos within capsules embedded in egg mass jelly (Burggren, 1985; Hunter and Vogel, 1986; Seymour and Roberts, 1991) may thus be one means of increasing the convective transport of oxygen through these egg masses (but see Strathmann and Strathmann, 1995).

Attempts to estimate the diffusion coefficient for oxygen transport across *Nucella* capsule walls have not been successful thus far (Rawlings, unpub. data). Nevertheless, two lines of evidence indicate that the diffusion coefficient of oxygen through capsule wall material may be lower than the diffusion coefficient of oxygen in water. First, although many neogastropod capsules are obviously permeable to small molecules such as water and salts, the rate of exchange of these molecules between capsules and their surrounding environment is slowed by the presence of the capsule wall (e.g., Pechenik, 1982; 1983; Roller and Stickle, 1989; Chapter 4). In fact, this slowed rate of exchange of salts appears critical to the ability of embryos of some species to tolerate large fluxes in salinity (see Pechenik, 1982; 1983). Second, in the present study, embryos within stripped capsules developed significantly faster than embryos in whole capsules (Fig. 7-7), thus suggesting that the outer capsule wall may be acting as a diffusive barrier to oxygen transport. Because *Nucella* capsules are relatively thin-walled (Pechenik, 1983; Rawlings, 1990;

1994; Chapter 2) compared to the thick gelatinous egg masses of other gastropods (e.g., Hurst, 1967; Strathmann and Strathmann, 1995), the protracted development of embryos with whole capsules appears unlikely to result from the fact that capsule walls are a thick, unstirred barrier to diffusion, such as egg mass jelly. Instead, constraints associated with the encapsulation of eggs within these thin-walled, fluid-filled capsules appear more likely to result from a low diffusion coefficient of the capsule wall to oxygen.

Aside from properties of the capsule wall, environmental conditions associated with habitats in which capsules or egg masses are spawned can have a profound effect on the development of embryos within these encapsulating structures. The exposure of *N. emarginata* capsules to moving water and air, for instance, shortened the development times of embryos considerably. Increased water motion, associated with a reduction in the thickness of the boundary layer surrounding capsules, reduced development times of embryos by ≈ 21% relative to those within capsules surrounded by still water (Fig. 7-10). Likewise, changes in the diffusion gradient of oxygen across the capsule wall, associated with the periodic emersion of capsules in air, resulted in embryonic development times that were ≈ 8% faster than those within capsules completely immersed in seawater (Fig. 7-9). Water motion and aeration have also been found to have dramatic effects on the developmental rate of eggs embedded within gelatinous egg masses of opisthobranch molluscs (see Strathmann and Strathmann, 1989, and references therein): embryos within masses exposed to faster moving water tend to exhibit greater developmental synchrony than those in slow moving water (Strathmann and Chaffee, 1984). Common behavioral responses by brooding females, such as fanning or drawing water across egg masses (see Hoagland, 1979; Giorgi and Congleton, 1984), further suggest that water motion may be very important for successful development of eggs within various types of encapsulating structures.

Relative to the effects of water motion and aerial exposure, however, the presence of the outer capsule wall had a surprisingly large influence on the rate of embryonic development. Embryos within stripped capsules of *N. emarginata* developed 10.1 - 13.9 % faster than those in whole capsules, under conditions of slow-moving water (Fig. 7-7). Although quantitative estimates of the boundary layer and capsule wall resistance to oxygen transfer were not determined in the present study, these results nevertheless suggest that both the capsule wall and boundary layer resistance may play an important role in constraining embryonic development within *N. emarginata* egg capsules. Thus direct measures of the resistance of the capsule wall and boundary layer to oxygen diffusion are now necessary to model oxygen transport across the walls of these benthic egg capsules. Such models should be instrumental in helping to understand the significance of packaging embryos within thick- versus thin-walled egg capsules.

A variety of techniques have been used successfully in conjunction with diffusion models to demonstrate constraints imposed on developing embryos by their egg coverings. For instance, Strathmann and Strathmann (1990) compared limitations associated with egg mass size and shape on the density of embryos packaged within gelatinous masses by embedding embryos within agar models. Likewise, by enclosing portions of egg masses within open-ended glass pipettes, they demonstrated the primary importance of oxygen limitation in retarding the development of embryos positioned within the centre of these masses (Strathmann and Strathmann, 1995). Novel techniques, such as these, however, have not been used previously to assess constraints associated with benthic, fluid-filled capsules.

Removal of the outer wall of muricid egg capsules (Rawlings, 1995; Chapter 5), however, appears to be an effective technique for demonstrating diffusive constraints associated with embryonic development within thick-walled *Nucella emarginata* capsules. This technique also looks promising for use in future studies. For instance, by assuming that stripped capsules have no wall resistance to oxygen transport, stripped and whole capsules can be used to estimate the wall resistance and total resistance (i.e., wall + boundary layer resistance) of capsules to oxygen diffusion, respectively. Also, by placing capsules under conditions of fast water movement, where boundary layers should be extremely thin, whole and stripped capsules can help to verify measures of the diffusivity of the capsule wall to oxygen. The ability to strip muricid capsules, therefore, may prove extremely useful in further examinations of diffusive constraints associated with fluid-filled egg capsules.

Costs and Benefits of Encapsulation

The encapsulation of eggs within benthic egg capsules can benefit developing embryos considerably. Egg capsules can provide embryos with at least some protection against predators (e.g., Strathmann, 1985; Pechenik, 1986; Perron, 1981; Rawlings, 1990; 1994; Rumrill, 1990), salinity stress (Pechenik, 1982, 1983), desiccation (Pechenik, 1978; Chapter 4) and possibly ultraviolet radiation (Chapter 5), as well as retain embryos within the parental habitat, and enclose embryos with a source of extraembryonic nutrition (Spight, 1976a; Rivest, 1983). Only now, however, are we beginning to understand some of the costs associated with the encapsulation of eggs, including constraints associated with the diffusive exchange of materials between embryos and their environment (Chaffee and Strathmann, 1984; Strathmann and Chaffee, 1984; this study), and the energetic costs of capsule production (Perron, 1981). The results of the present study demonstrate that the walls of *Nucella emarginata* egg capsules may indeed impose significant constraints on embryonic development.

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Table 7-1. Log-log regression equations describing associations between the number of hatchlings per capsule chamber and the chamber volume (in µl) for *N. emarginata* capsules collected from ten sites in Barkley Sound. Sample size (N) refers to the number of clutches sampled per site. Each clutch represents the mean of 5-6 egg capsules.

SITE	X-variable	N	r^2	Slope (±SE)	P	Intercept (±SE)	RMA Slope[a]
Grappler	Log (Volume)	10	0.644	0.696 (0.1830)	0.005	0.292 (0.2712)	0.867[b]
Ross Islet	Log (Volume)	10	0.080	-0.958 (1.1499)	0.429	2.435 (1.4621)	-------
Kelp Bay	Log (Volume)	10	0.300	0.756 (0.4080)	0.101	0.334 (0.4749)	-------
Dixon Island	Log (Volume)	10	0.176	0.711 (0.5435)	0.227	0.259 (0.6669)	-------
Self Point	Log (Volume)	10	0.031	0.275 (0.5473)	0.629	0.773 (0.6004)	-------
Wizard Rock	Log (Volume)	10	0.042	0.183 (0.3076)	0.569	0.853 (0.3699)	-------
Voss Point	Log (Volume)	10	0.558	1.035 (0.3256)	0.013	-0.166 (0.3818)	1.385[b]
Kirby Point	Log (Volume)	10	0.412	1.070 (0.4517)	0.045	-0.252 (0.5825)	1.667[b]
Cape Beale	Log (Volume)	10	0.479	1.231 (0.4539)	0.027	-0.534 (0.6252)	1.779[b]
Folger Island	Log (Volume)	11	0.629	1.254 (0.3208)	0.004	-0.545 (0.4057)	1.581[b]

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[a] Because there was error associated with both the y- and x-axis, a Model II (RMA) regression was used to provide an estimate of the scaling exponent for these relationships (see McArdle, 1988; Hess, 1993).

[b] None of these RMA slopes differed significantly from a slope of 1.0

Figure 7-1. Respirometer designed to measure the oxygen consumption of embryos within whole and stripped egg capsules of *Nucella emarginata*.

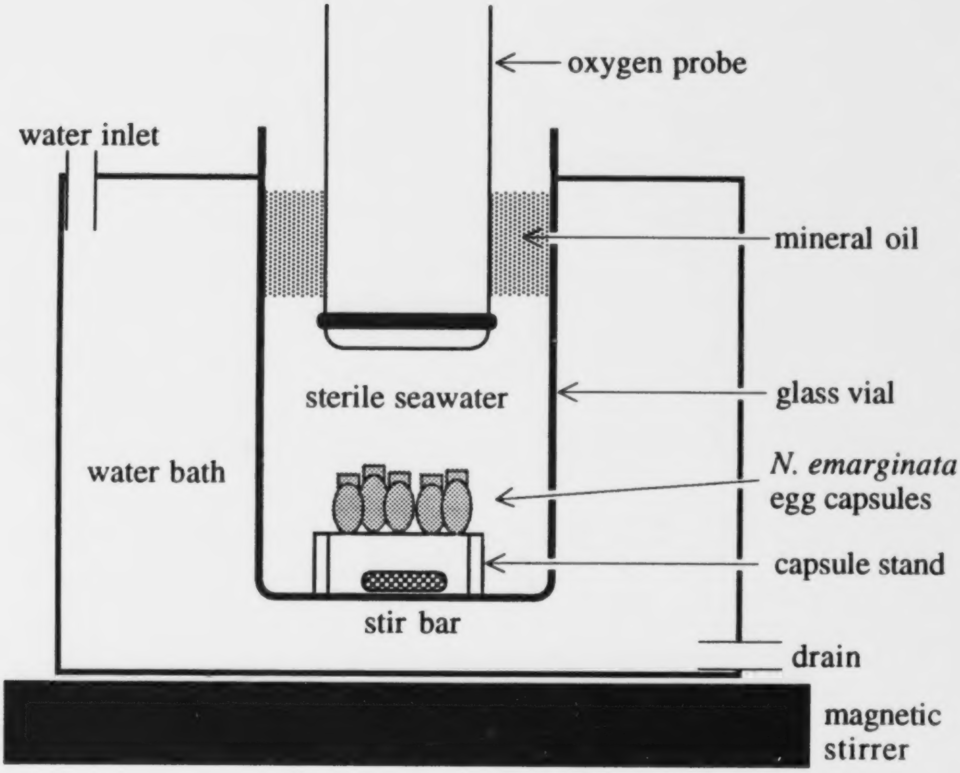
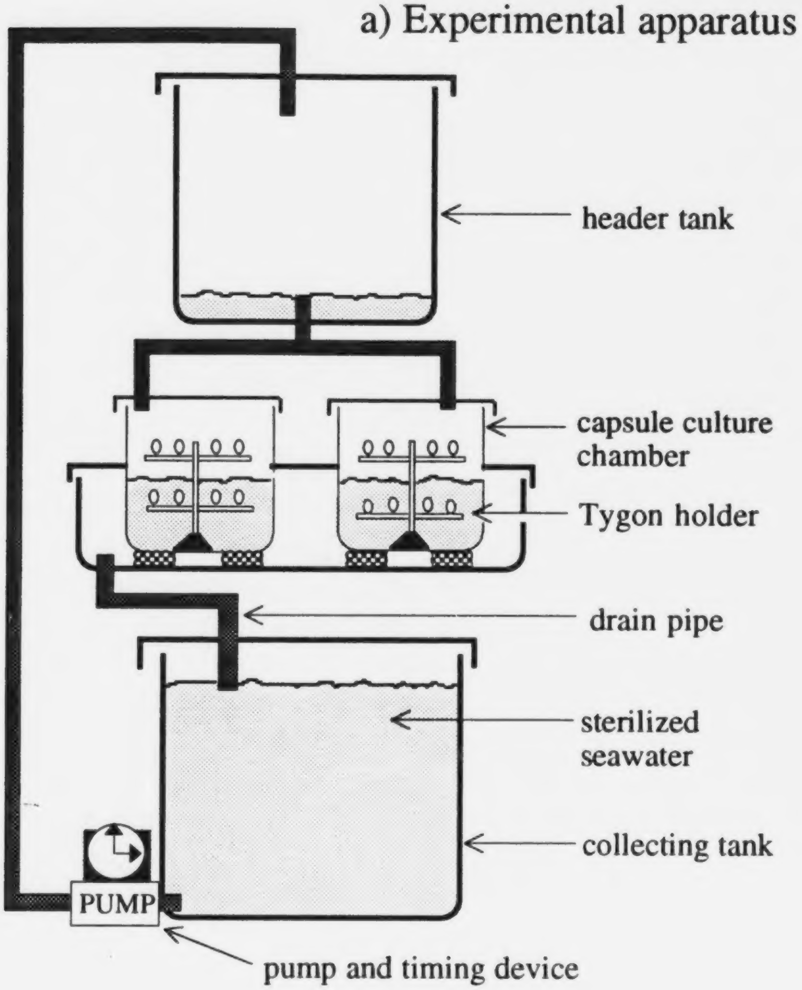
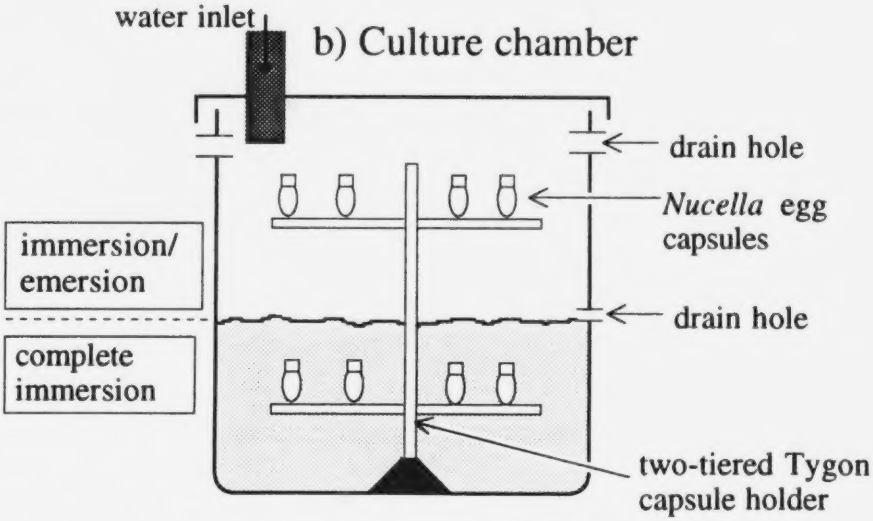

oxygen probe
water inlet
mineral oil
sterile seawater
glass vial
water bath
N. emarginata egg capsules
capsule stand
stir bar
drain
magnetic stirrer

Figure 7-2. Experimental apparatus used to expose *Nucella emarginata* egg capsules to either a complete immersion cycle (12 / 12 h completely immersed) or a immersion / emersion cycle (6 / 12 h immersed, 6 / 12 h emersed). Shown are: a) the whole set-up including a water pump, a header tank, two culture chambers, a collecting tank and a timing device, and b) the two tiered capsule holder used to suspend paired capsules at two different heights within the culture chambers.


a) Experimental apparatus

header tank
capsule culture chamber
Tygon holder
drain pipe
sterilized seawater
collecting tank
PUMP
pump and timing device


b) Culture chamber

water inlet
drain hole
Nucella egg capsules
immersion/ emersion
complete immersion
drain hole
two-tiered Tygon capsule holder

Figure 7-3. Intraspecific variation in the morphology of *Nucella emarginata* capsules, and the packaging of embryos within capsules, among ten intertidal populations in Barkley Sound. Each histogram represents the mean (± SE) of 10 - 11 clutches sampled per population (five to six capsules averaged per clutch). Differences in capsule form were compared among populations using ANOVA. The results of Tukey *a posteriori* multiple comparison tests ($\alpha = 0.05$) are shown in the inset above each graph. Abbreviations for each site refer to: GR - Grappler Inlet; RI - Ross Islet; KB - Kelp Bay; DX - Dixon Island; SP - Self Point; WZ - Wizard Rock; VP - Voss Point; KP - Kirby Point; CB - Cape Beale; FG - Folger Island. Sites are ranked along the x-axis in order of increasing wave-exposure.

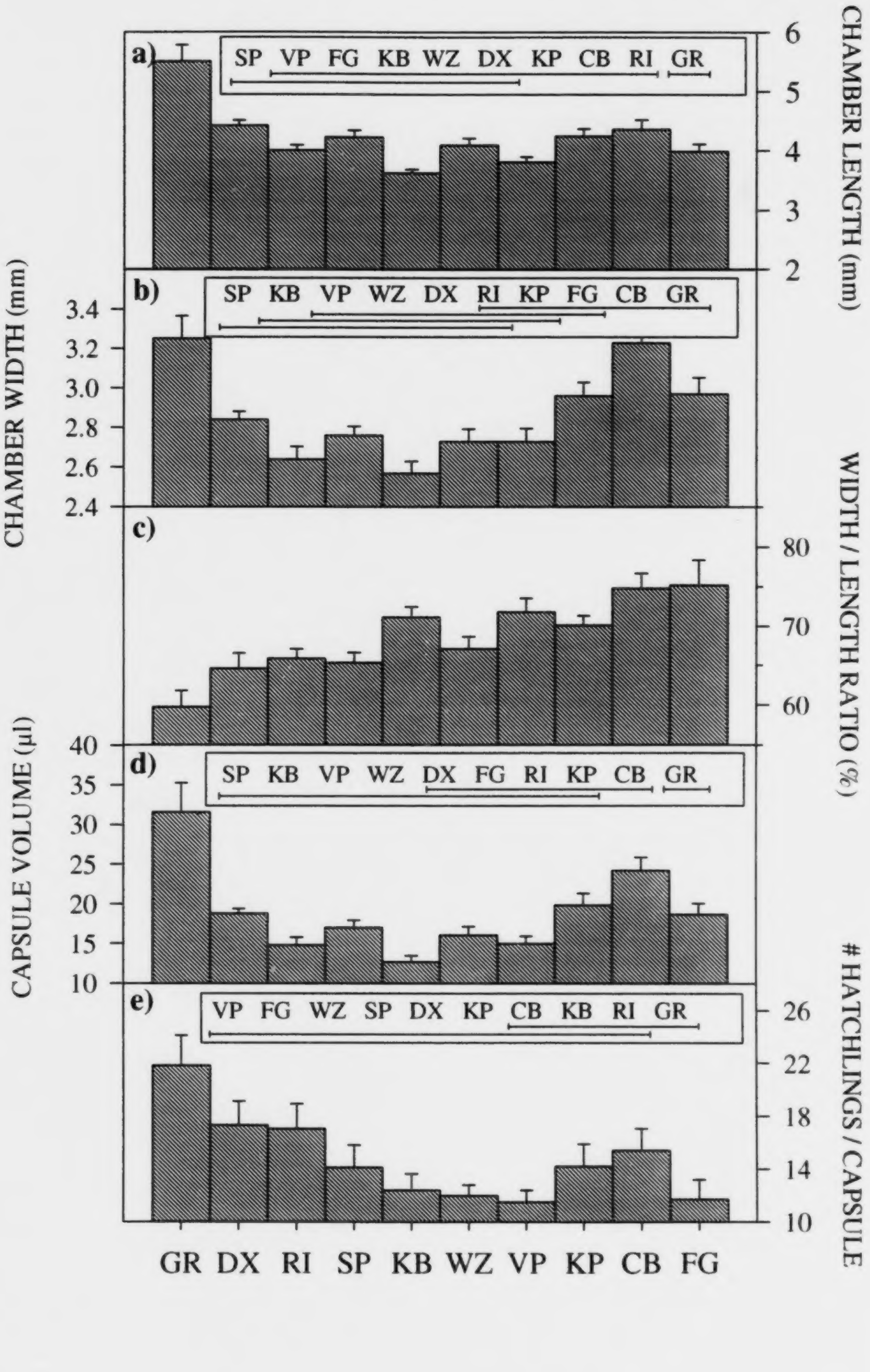

a)
SP VP FG KB WZ DX KP CB RI GR
CHAMBER LENGTH (mm)
2
3
4
5
6
b)
SP KB VP WZ DX RI KP FG CB GR
CHAMBER WIDTH (mm)
2.4
2.6
2.8
3.0
3.2
3.4
c)
WIDTH / LENGTH RATIO (%)
60
70
80
d)
SP KB VP WZ DX FG RI KP CB GR
CAPSULE VOLUME (µl)
10
15
20
25
30
35
40
e)
VP FG WZ SP DX KP CB KB RI GR
# HATCHLINGS / CAPSULE
10
14
18
22
26
GR DX RI SP KB WZ VP KP CB FG

Figure 7-4. Among-site differences in the number of hatchlings per unit chamber volume for ten populations of *Nucella emarginata* in Barkley Sound. Each symbol represents the mean (± SE) of 10 - 11 clutches of capsules sampled per population (5 - 6 capsules averaged per clutch). Abbreviations for each site are given in Figure 7-3. Mean hatchling / volume ratios were compared among sites using ANOVA. The results of Tukey *a posteriori* multiple comparison tests ($\alpha = 0.05$) are shown in the inset above the graph.

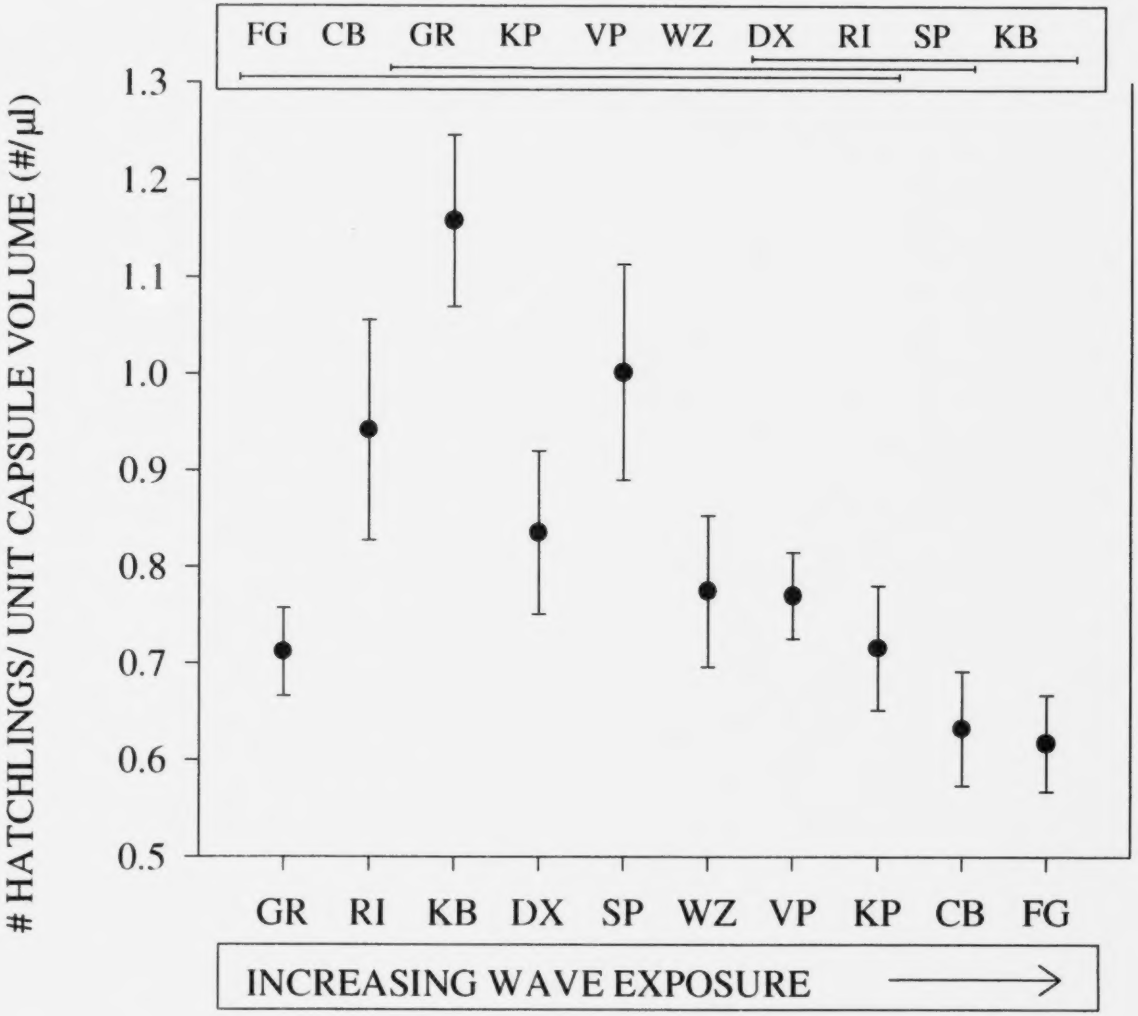

FG CB GR KP VP WZ DX RI SP KB
# HATCHLINGS/ UNIT CAPSULE VOLUME (#/µl)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
GR
RI
KB
DX
SP
WZ
VP
KP
CB
FG
INCREASING WAVE EXPOSURE

Figure 7-5. Associations between the number of embryos per capsule and the chamber volume for ten populations of *Nucella emarginata*. Each symbol refers to the mean number of embryos and mean capsule volume for 5 - 6 capsules sampled per clutch; 10 - 11 clutches were sampled per population. Hollow symbols represent capsules collected from sites with thick capsule walls: Grappler Inlet, Kirby Point, Cape Beale, and Folger Island (n = 41). Filled symbols represent capsules from sites with relatively thin capsule walls: Ross Islet, Dixon Island, Kelp Bay, Self Point, Wizard Rock and Voss Point (n = 60).

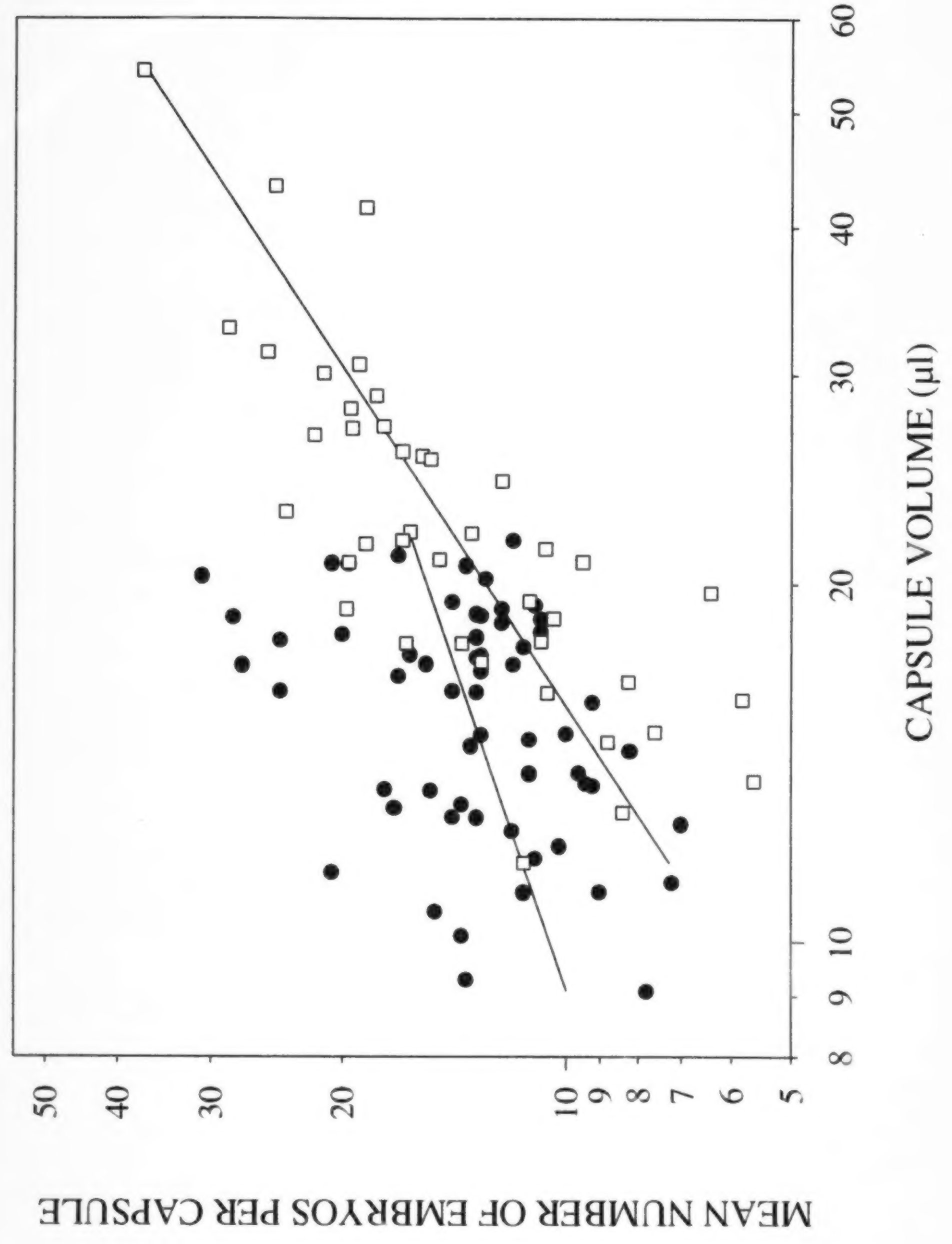

MEAN NUMBER OF EMBRYOS PER CAPSULE
50
40
30
20
10
9
8
7
6
5
CAPSULE VOLUME (µl)
8
9
10
20
30
40
50
60

Figure 7-6. Associations between the mean number (± SE) of embryos per unit capsule volume and mean capsule wall thickness (± SE) among ten study populations of *Nucella emarginata* from Barkley Sound. Ratios of embryo number per unit capsule volume were based on the average of five - six capsules sampled from each of 10 - 11 clutches per population. Estimates of capsule wall thickness were based on average of three capsules sampled from each of ten clutches per site. The least-squares linear regression equation for this relationship was significant: $Y = -0.0091 X (\pm 0.00273) + 1.4548 (\pm 0.19493)$, $r^2 = 0.582$, $P < 0.01$, $n = 10$. Also shown in the inset is the mean wall thickness (± SE) of capsules collected from each population, based on the sample sizes given above. The mean wall thickness of capsules was compared among sites using ANOVA. The results of Tukey *a posteriori* multiple comparison tests ($\alpha = 0.05$) are shown above the graph.

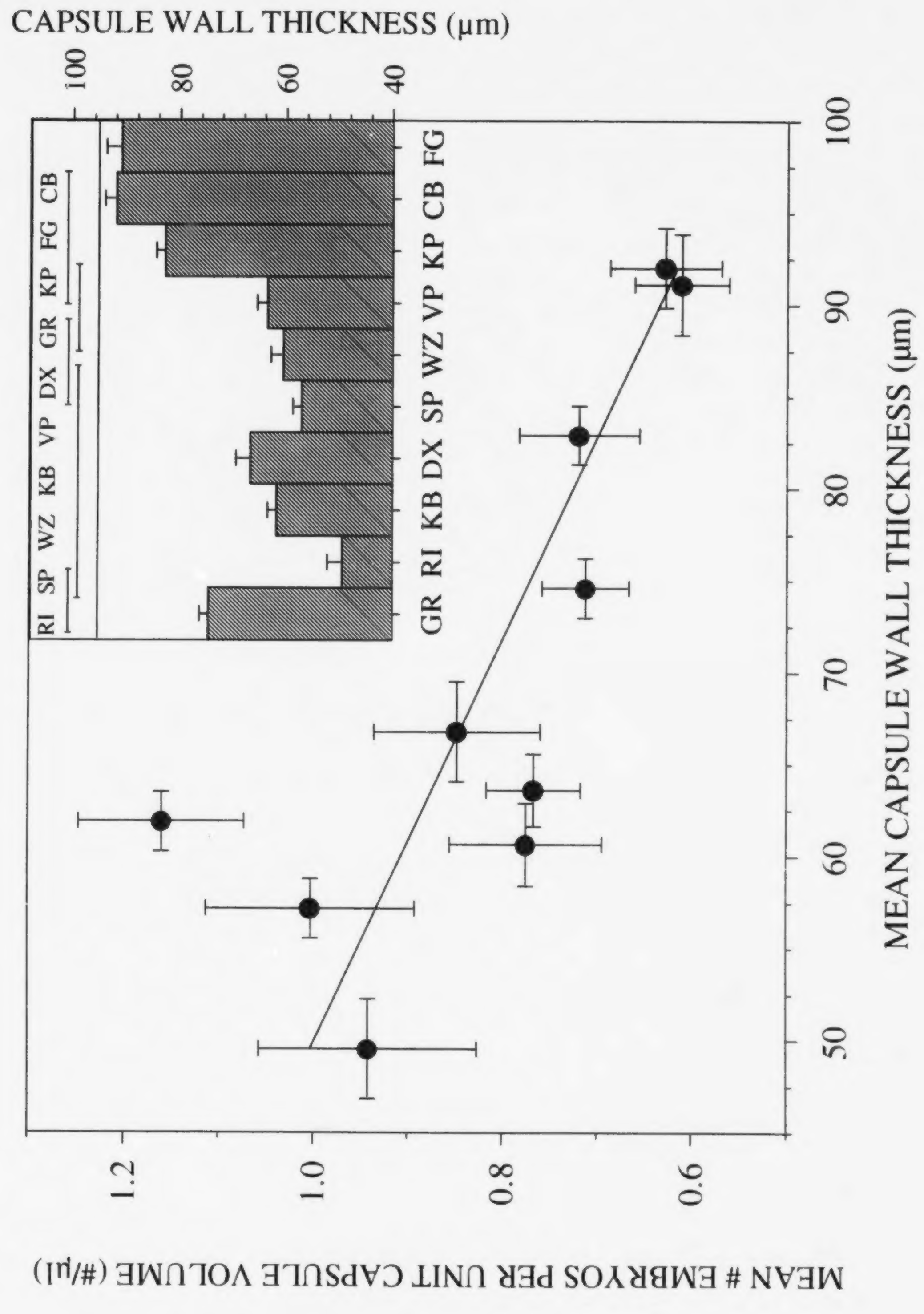

CAPSULE WALL THICKNESS (µm)
100
80
60
40
RI SP WZ KB VP DX GR KP FG CB
GR RI KB DX SP WZ VP KP CB FG
MEAN # EMBRYOS PER UNIT CAPSULE VOLUME (#/µl)
1.2
1.0
0.8
0.6
MEAN CAPSULE WALL THICKNESS (µm)
50
60
70
80
90
100

Figure 7-7. The development time of embryos within whole capsules versus siblings within capsules stripped of their outer wall in two separate experiments. Each symbol represents the results for paired stripped and whole capsules. The diagonal line represents an equal rate of development for embryos within whole and stripped capsules. Symbols falling above the line indicate that embryos within whole capsules took longer to develop. Symbols falling below the line indicate that embryos within stripped capsules took longer to develop. Because the actual date that capsules were spawned may have been 1-2 weeks prior to allocating capsules to treatment conditions, development times dc not necessarily reflect the total encapsulated development time of this species. The flagged symbol represents the only pair of capsules in which embryos within the whole capsule emerged before embryos within the stripped capsule. Because the delayed hatching of embryos within this stripped capsule resulted from the occlusion of the capsular plug with a hatching snail, the development time of embryos within these two capsules was not included in the statistical analysis of the results. A paired t-test on data pooled for both years indicated that development times were significantly longer in whole capsules relative to stripped capsules: Paired t-test: $t = 6.79$, $P < 0.001$, $n = 22$)

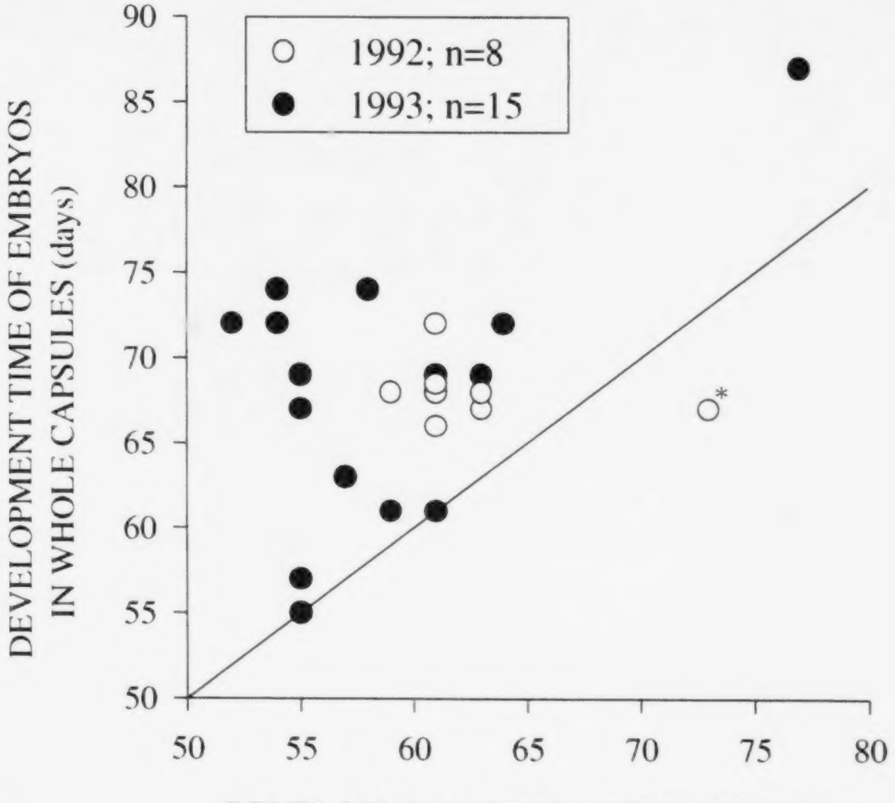


Figure 7-8. The relationship between the mean consumption of oxygen per *N. emarginata* embryo (± SE, n = 5 trials) and the partial pressure of oxygen within the experimental chamber, for embryos within whole capsules and capsules stripped of their outer wall. Experiments were conducted at 12°C. Comparison of the metabolic rates of embryos within whole and stripped capsules over PO_2 ranges from 140 - 120, 120 -100, 100 - 80, 80 - 60 and 60 - 40 Torr, indicated that embryos within stripped capsules had faster metabolic rates over each range (paired t-test, t = 3.22; P = 0.032).

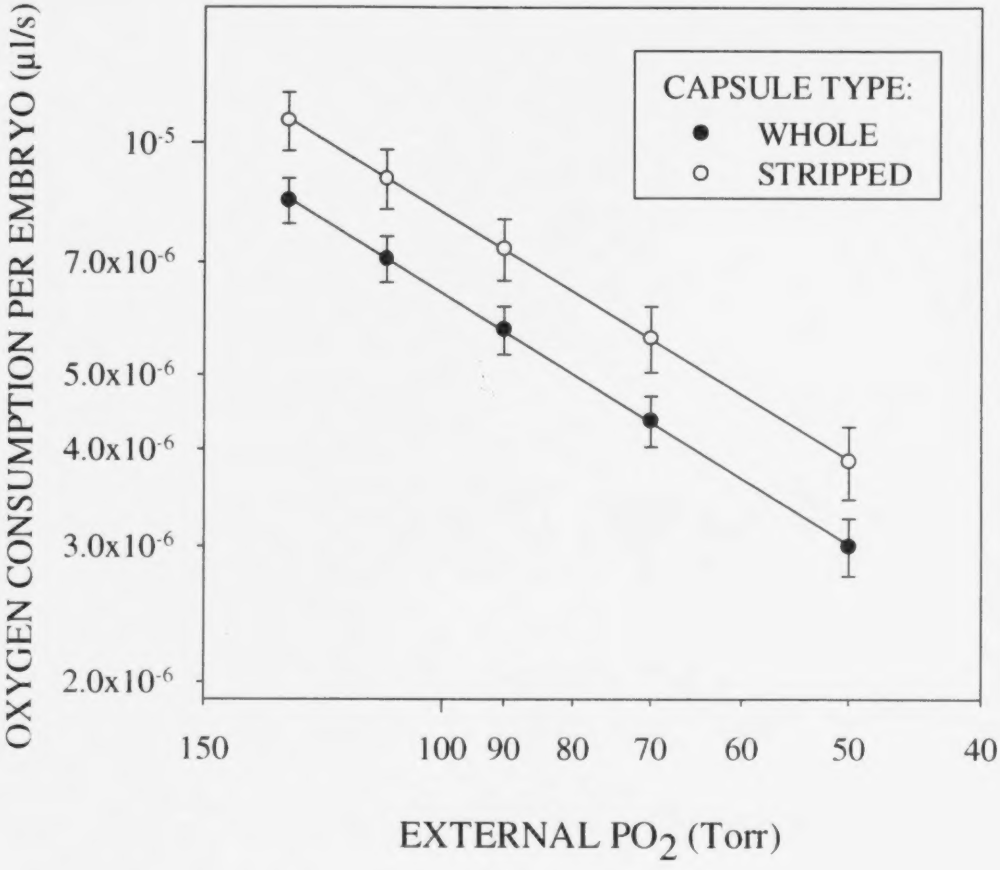


Figure 7-9. The development time of encapsulated embryos of *Nucella emarginata* when kept completely immersed in seawater over a 12 h cycle, or exposed to 6 h of immersion and 6 h of emersion at 12°C. Each symbol represents the results of paired capsules (i.e., from the same clutch) assigned to each treatment. Symbols falling along the diagonal line indicate equal rates of development of encapsulated embryos within both treatment conditions. Symbols falling above the line indicate that embryos within the complete immersion treatment took longer to develop. Symbols falling below the line indicate that embryos within the immersion / emersion treatment took longer to develop. Because the actual date that capsules were spawned may have been 1-2 weeks prior to allocating capsules to treatment conditions (Day 0), development times do not necessarily reflect the total encapsulated development time of this species.

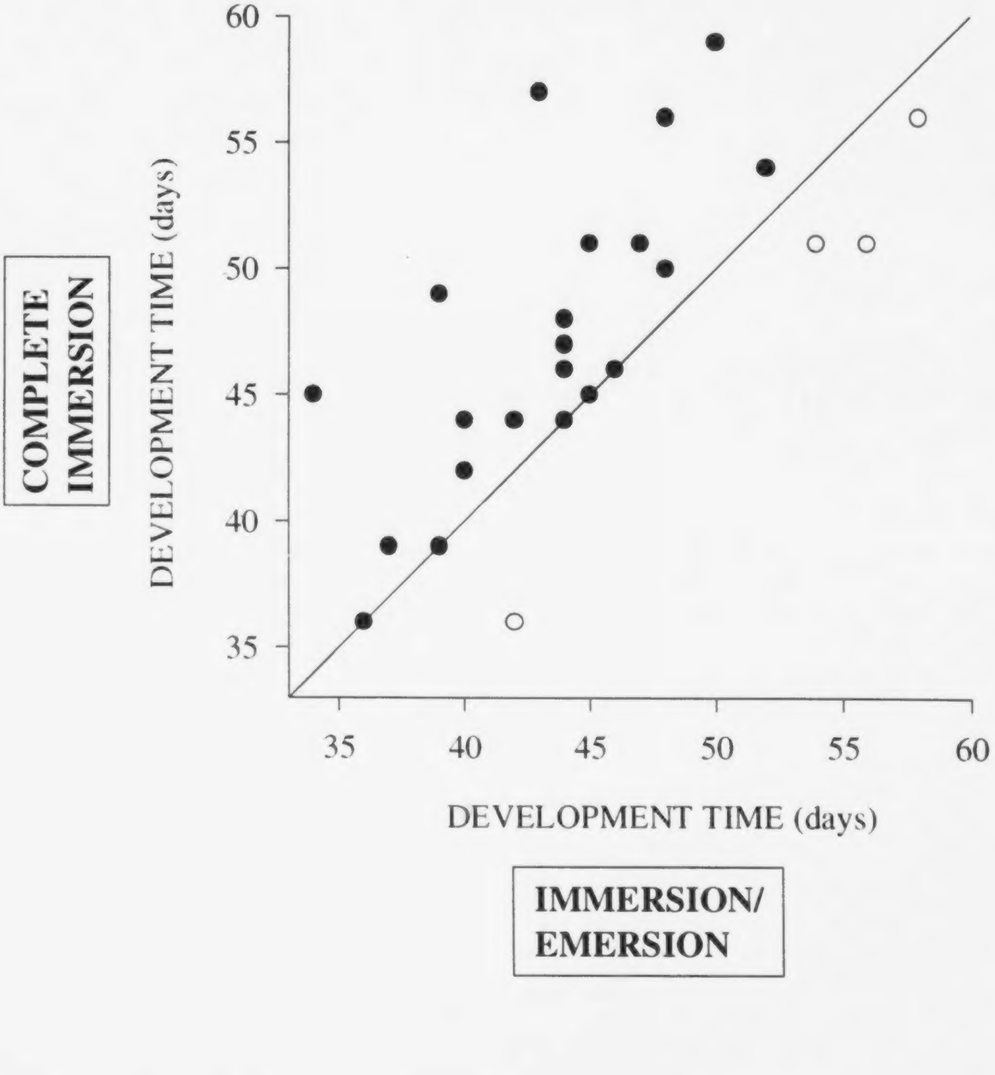
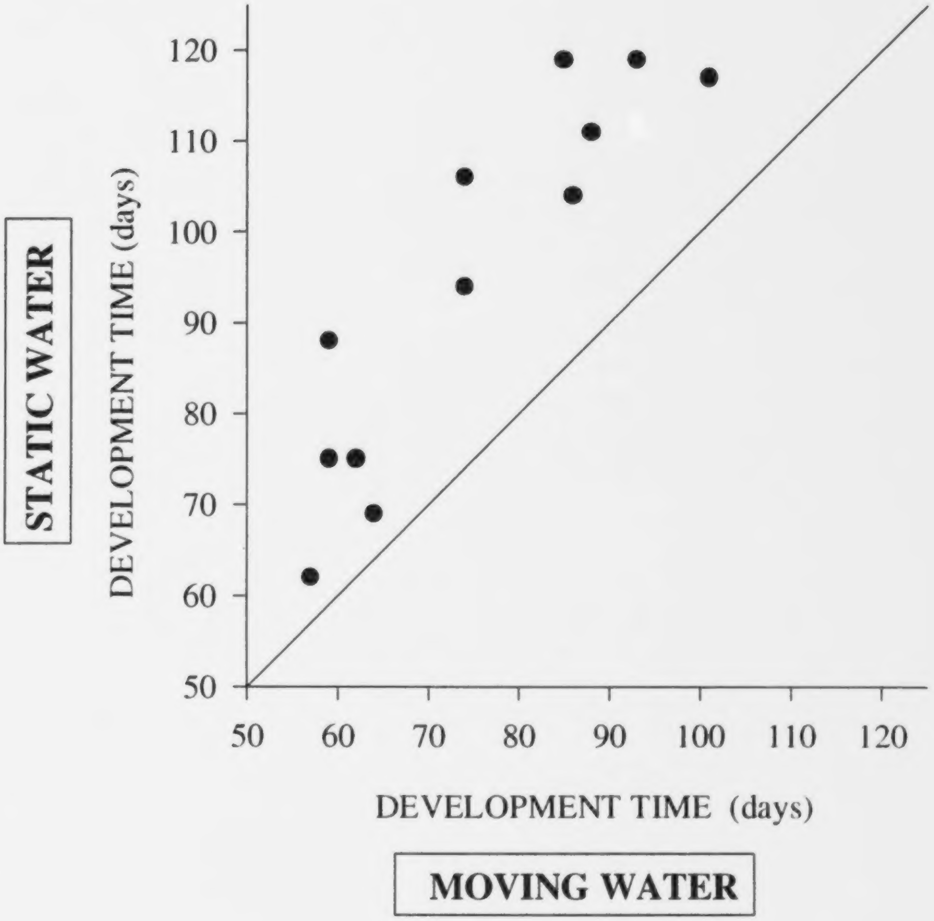


Figure 7-10. Development time of encapsulated embryos of *Nucella emarginata* exposed to conditions of still and moving water at 10 - 11°C. Each symbol represents the results of paired capsules (i.e., from the same clutch) assigned to each treatment. Symbols falling above the line indicate that embryos within the static water treatment took longer to develop. Symbols falling below the line indicate that embryos within the moving water treatment took longer to develop. Because the actual date that capsules were spawned may have been 1-2 weeks prior to allocating capsules to treatment conditions (Day 0) , development times do not necessarily reflect the total encapsulated development time of this species.



CHAPTER 8

General Conclusions

The evolution of egg coverings within the Gastropoda

Although gastropod phylogeny is only coarsely resolved (see Haszprunar, 1988; Bieler, 1992), the production of egg coverings is undoubtedly a derived condition within this group. Despite considerable debate as to whether ancestral gastropod larvae were planktotrophic or lecithotrophic (see Page, 1994), these larvae were likely to have been free-living (Thorson, 1950; Strathmann, 1978; Strathmann, 1985; Chaffee and Lindberg, 1986). Support for this is provided by the absence of egg coverings among extant groups of lower prosobranch gastropods. Patellogastropod limpets, for instance, a sister group to all other gastropods, exhibit a transition from broadcast spawning to brooding eggs as one progresses from ancestral to derived taxa (see Lindberg, 1988), but none encapsulate their eggs with glandular secretions of the reproductive tract (but see Kessel, 1964). Likewise, lower archaeogastropods typically have simple reproductive systems, with broadcast spawning, external fertilization, and short-lived, free-swimming, non-feeding larvae (Hadfield and Strathmann, 1990; Hickman, 1992). Hence, ancestral prosobranch gastropods were likely broadcast spawners, with no means of encapsulating their eggs.

Because egg coverings have appeared in the higher vetigastropods, one subclade of the Archaeogastropoda (Lindberg and Ponder, 1991), and separately in the Caenogastropoda, they have probably evolved at least twice within the Gastropoda. Within the Vetigastropoda, for instance, some trochoidean snails produce a variety of gelatinous egg coverings surrounding their eggs (Fretter and Graham, 1962; Hadfield and Strathmann, 1990; Hickman, 1992). Unlike the egg coverings of caenogastropods, opisthobranchs and pulmonates, these egg coverings are derived from the ovary and urogenital papilla (Fretter and Graham, 1962; Hickman, 1992), rather than specialized glands in the reproductive tract. Thus, not only do these encapsulating materials appear to be a unique derived feature of trochoidean snails, but they are also unlikely to be homologous to the egg capsules and masses of higher gastropods (Hickman, 1992). Since current phylogenies indicate that trochoidean snails and caenogastropods do not share a common ancestor that produced egg coverings (but see Hadfield and Strathmann, 1990), this suggests that encapsulated development has evolved at least twice within these early gastropod groups.

Given that egg coverings have evolved more than once within the Gastropoda, and also independently in other phylogenetic groups (see Jägersten, 1972), these structures seem likely to be adaptive. Adaptive explanations concerning the evolution of benthic masses and capsules have generally focused on the lower vulnerability of encapsulated embryos to predation and starvation within benthic habitats compared to unprotected larvae within the plankton (e.g., Vance, 1973, Pechenik, 1979; Grant, 1980; Strathmann, 1985). Indeed, there is a mounting body of evidence to indicate that instantaneous mortality rates may be considerably higher for free-living larvae within the plankton compared to those within benthic egg capsules (Strathmann, 1985, Rumrill, 1990). Although this adaptive explanation provides one mechanism for the evolution of encapsulation, it does not explain the tremendous diversity in the form of encapsulating structures that exists within families of gastropods, among closely-related species, and even among populations of species. What then are the causes of this variation?

Adaptive significance of variation in the form of gastropod egg coverings

One way to understand the adaptive significance of differences in the form of gastropod egg coverings is through the joint mapping of spawn characteristics and other life-history and ecological attributes onto an independently derived cladogram of the Gastropoda, thus enabling a formal comparative test of adaptive explanations (sensu Harvey and Pagel, 1991). The lack of a rigorously constructed phylogeny for the Gastropoda, however, has thwarted attempts to examine the evolution of spawn within this group as a whole. Nevertheless, cladistic analyses of relationships within and among gastropod genera have helped to map the direction of spawn evolution within specific groups (see Reid, 1990, 1991; Collins et al., in review). For instance, within *Neritrema*, a subgenus of littorine snails, primitive pelagic egg capsules were first embedded in a protective benthic gelatinous mass. These capsules were then gradually reduced or lost, eventually resulting in a derived condition in which the eggs were retained entirely within the oviduct (see Reid, 1990). Such knowledge of the ancestral and derived forms of spawn within a group is essential to testing adaptive hypotheses about the evolution of different spawn morphologies (Harvey and Pagel, 1991).

Attempts to provide adaptive explanations for derived forms of gastropod egg coverings, even within closely-related groups of gastropods, have only met with a modicum of success, however. Often, because only general information is available on the life-histories and habitat types of many gastropod species, comparative tests of adaptive explanations have relatively low resolving power (e.g., Reid, 1990). Support for the tentative conclusions of such comparative tests have also been lacking because so few studies have independently tested the adaptive value of specific capsule traits. Given the diversity of gastropod spawn, our ignorance is staggering. We know virtually nothing, for instance, about the costs and benefits of enclosing eggs within such markedly different types of egg coverings as gelatinous masses versus fluid-filled capsules, let alone the consequences of packaging eggs within multilaminated capsules versus simple forms, smooth versus elaborately sculptured capsules, or pelagic versus benthic capsules. Thus, given our poor understanding of the functional benefits of such egg coverings, simply knowing the direction of spawn evolution and associated changes in environmental conditions may not be enough to interpret the adaptive significance of subtle changes in spawn morphology.

The ground swell of interest in using cladistic relationships to infer adaptive explanations for the evolution of gastropod egg coverings thus must be balanced with studies of the costs and benefits of different spawn types. If variation in the form of gastropod egg coverings does reflect adaptive responses to a suite of new selective pressures within benthic marine habitats (Pechenik, 1986), then direct evidence should exist. Indeed, the results of my thesis provide strong evidence to suggest that intraspecific variation in some components of *N. emarginata* capsules, most specifically capsule wall thickness, are adaptive responses to specific environmental conditions. For instance, changes in capsule wall thickness significantly affected the vulnerability of encapsulated embryos to intertidal predators (Chapter 3) and ultraviolet radiation (Chapter 5). Likewise, thicker walls also slowed rates of water loss from capsules under certain environmental conditions, although this effect was not large relative to variation in other properties of capsular cases (Chapter 4). Experimental removal of the outer capsule wall also illustrated the importance of specific wall laminae in protecting embryos from attack by intertidal isopods (Chapter 3), protists and bacteria (Chapter 6) and in absorbing harmful ultraviolet radiation (Chapter 5). Thus, because 1) capsule wall thickness varies substantially among populations of *N. emarginata*, 2) this variation appears to have some underlying genetic basis (Chapter 2), and 3) there are obvious fitness-related benefits associated with changes in capsule wall thickness (Chapters 3, 4, 5), thicker walled capsules likely represent an adaptive response to specific environmental risks. Given the apparent underlying genetic basis associated with interpopulation variation in capsule body length and relative plug length (Chapter 2), variation in these features of *N. emarginata* capsules may also have an adaptive basis.

Interpretations of the evolutionary significance of variation in the morphology of egg coverings may also be enhanced by understanding the constraints associated with the packaging of eggs within these structures (e.g., Chaffee and Strathmann, 1984, Strathmann and Chaffee, 1984). Such constraints can be identified by means of direct tests or manipulations of the form of gastropod egg capsules and masses. For instance, direct manipulation of the wall thickness of *N. emarginata* capsules illustrated that thicker-walled capsules resulted in the prolonged development of encapsulated embryos, possibly due to constraints associated with the diffusion of oxygen or metabolic wastes across the capsule wall (Chapter 7). Likewise, comparisons of the numbers of embryos within different capsule morphs indicated that thicker walled capsules were associated with lower numbers of embryos per unit capsule volume than thinner walled capsules (Chapter 7). Evolutionary changes in capsule form may thus be limited by physical constraints associated with the packaging of embryos within these structures.

Future directions

Clearly, much remains to be learned about the diverse array of egg coverings produced by marine gastropods. Ultimately our success in understanding the evolutionary significance of these diverse spawn types will depend on directing our efforts in three main directions. First, we must try to resolve the relationships within and between gastropod families so that we can document the sequence of spawn evolution within the Gastropoda. Given the advent of powerful new molecular techniques for inferring relationships between gastropod species, the future looks promising in this regard (e.g., Tillier et al., 1992; Rosenberg et al., 1994). Second, we must develop an extensive database documenting ecological, and life-history characteristics of marine gastropods, so that these features can be mapped onto cladistic analyses of their relationships. Although it appears that this is being done for some groups (e.g., D'Asaro, 1991; 1993; Reid, 1990; Hickman, 1992; Collins et al., in prep.), a more concerted effort is needed to make this a global venture. Third, we must begin in earnest to conduct experimental tests of the costs and benefits of differences in capsule form. As in the present study, such tests can be instrumental in identifying which components of capsule form may have an adaptive basis.

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APPENDIX 1

Supplementary figures to Chapter 2

Figure A1-1. Mean dry weights of cases for three different capsule wet weights (0.02, 0.025, 0.030 g) for all ten study populations. Means were based on the regression equations given in Table 2-3. Collection sites are ranked on the X-axis according to increasing capsule wall thickness (see Fig. 2-4).

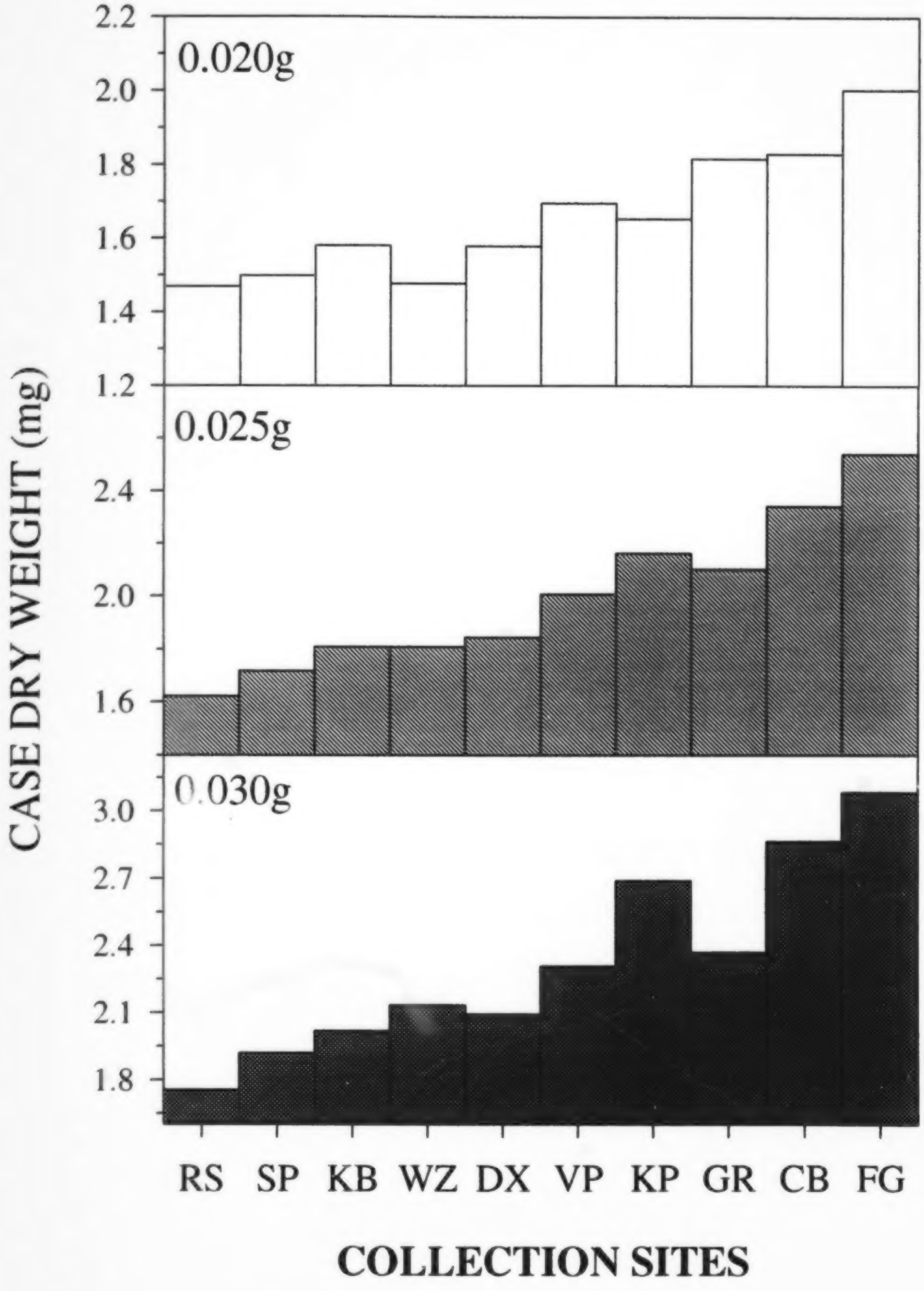
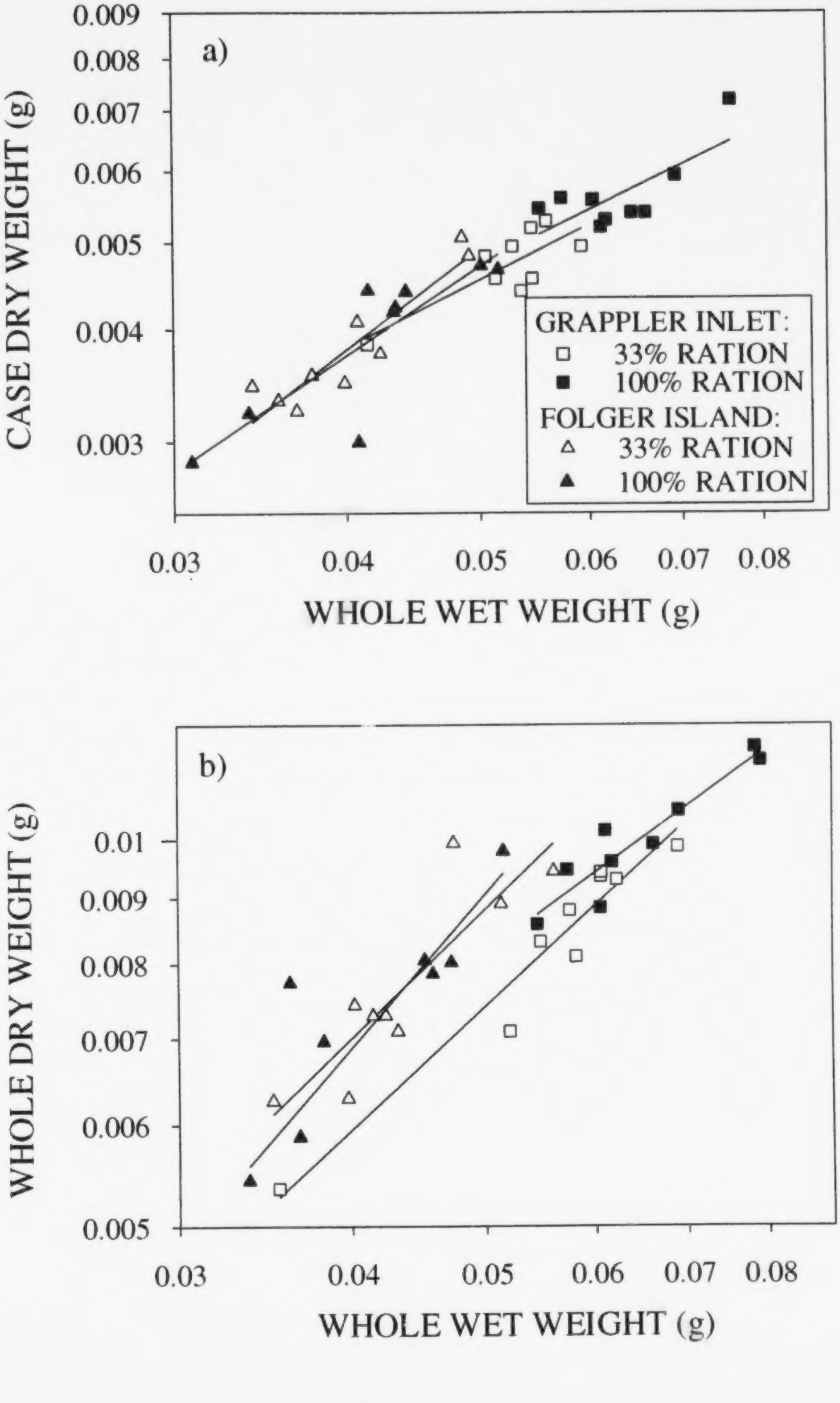

0.020g
0.025g
0.030g
CASE DRY WEIGHT (mg)
2.2
2.0
1.8
1.6
1.4
1.2
2.4
2.0
1.6
3.0
2.7
2.4
2.1
1.8
RS
SP
KB
WZ
DX
VP
KP
GR
CB
FG
COLLECTION SITES

Figure A1-2. Relationships between a) log (case dry weight) and log (whole wet weight) and b) log (whole dry weight) and log (whole wet weight), for capsules produced by laboratory populations of Grappler Inlet and Folger Island snails following > 6 months exposure to two different ration treatments: 33% ration and 100% ration. Results from Ross Islet snails are not included because too few capsules were spawned by snails surviving these treatment conditions. See Table 2-7 for actual sample sizes and regression equations.


a)
CASE DRY WEIGHT (g)
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.03
0.04
0.05
0.06
0.07
0.08
WHOLE WET WEIGHT (g)
GRAPPLER INLET:
□ 33% RATION
■ 100% RATION
FOLGER ISLAND:
△ 33% RATION
▲ 100% RATION
b)
WHOLE DRY WEIGHT (g)
0.01
0.009
0.008
0.007
0.006
0.005
0.03
0.04
0.05
0.06
0.07
0.08
WHOLE WET WEIGHT (g)

APPENDIX 2

Supplementary equations used in Chapter 5

1. Concentration Gradient in Water Vapor Density Across the Capsule Wall

To measure the concentration gradient in water vapor density across the capsule wall, it was necessary to estimate the water vapor density of air at the capsule surface, as well as the water vapor density of the surrounding air.

a)Vapor density of air at the capsule surface

I assumed that at the surface of the capsule chamber, the vapor density was equivalent to that of saturated air, corrected for salinity. The vapor pressure of pure water at saturation was calculated from air temperature using the following relationship from Campbell (1977):

$$\text{vapor pressure of pure water } (p_w) = \exp(52.58 - (6790.5/T) - 5.028 \ln(T) \qquad \textbf{(1)}$$

where T is the air temperature in °K. The vapor density of pure water was calculated as:

$$\text{vapor density } (\rho') = 10{,}000\ p\ /\ (4.62\ T) \qquad \textbf{(2)}$$

Because capsules were filled with seawater, however, the vapor pressure of pure water had to be corrected for that of seawater at 32‰. The vapor pressure of seawater is related to that of pure water by the equation (Harvey, 1955):

$$\text{vapor pressure of seawater } (p_{sw}) = p_w\ (1 - 0.00097\ \text{Cl‰}) \qquad \textbf{(3)}$$

where Cl‰ is the chlorinity of seawater. Chlorinity is related to salinity in the following equation: salinity = 0.03 + 1.805 (Chlorinity) (Arons and Kientzler, 1954). Assuming a chlorinity of ≈ 17.7‰ at a salinity of 32‰, seawater has a 1.8% reduction in vapor pressure relative to pure water. The saturation vapor density at the capsule surface was thus calculated as:

$$\text{saturation vapor density } (\rho'_{sw}) = 10{,}000\ p_{sw}\ /\ (4.62\ T) \qquad \textbf{(4)}$$

b)Vapor density of air surrounding egg capsules

The vapor density of air in the ambient environment was determined using the following equation (Campbell, 1977):

$$\rho_v = \rho'\ (RH) \qquad \textbf{(5)}$$

where ρ' is the vapor density of saturated air at ambient temperature, as defined above, and RH is the relative humidity of the air. The relative humidity of air in this study was measured using a portable thermohygrometer.

2. Changes in Water Vapor Density at the Capsule Surface with Increasing Dehydration

The loss of water from the capsule chamber can have an increasingly large effect on the water vapor density of air at the capsule surface. Because the loss of water is associated with an increase in salt concentration within the capsule, and because the concentration of salts can affect the vapor pressure of saturated air, the gradient of water vapor density declines with a loss of water from the capsule chamber. A loss of 50% of the volatile water from the capsule chamber can result in a doubling of the concentration of salts. Using equation (3), therefore, this results in a 3.4% reduction in vapor pressure relative to pure water. The effect of salt concentration on the vapor pressure at the capsule surface thus becomes increasingly large as less as less water remains in the capsule chamber. In this study, however, because in most experimental trials capsules lost only ≈50% of their volume, which represented a change of ≤ 3.4% in the vapor pressure gradient, I did not correct for the effect of increased salinity within the capsule chamber.

3. Calculation of the Reynolds Number

The Reynolds number is a dimensionless term that characteristics the flow of a fluid about an object. Is is described by the following equation (Vogel, 1981):

$$Re = (\rho \; l\, U)/ \mu \qquad \textbf{(6)}$$

where ρ is the dynamic viscosity of the fluid, l is the characteristic length of an object parallel to flow, U is the velocity of the fluid, and μ is the density of the fluid. This equation can be rewritten as:

$$Re = (l\, U)/ v \qquad \textbf{(7)}$$

where v is the kinematic viscosity of the fluid (i.e., $\rho \; / \mu$).

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